University of Alberta

Novel HPLC separation methodologies compatible with chemiluminescent nitrogen detection

by

Christopher Robin Harrison ©

A thesis submitted to the Faculty of Graduate Studies in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Department of Chemistry

Edmonton Alberta

Fall 2005

Library and Archives Canada

Published Heritage Branch

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque et Archives Canada

Direction du Patrimoine de l'édition

395, rue Wellington
Ottawa ON K1A 0N4
Canada

0-494-08652-1

Your file Votre référence
ISBN:
Our file Notre reterence
ISBN:

NOTICE:
The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

AVIS:
L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

Canada

"I thought of it while riding my bicycle."

-Albert Einstein, on the theory of relativity.

ABSTRACT

The methodology of high performance liquid chromatography (HPLC) separations has been well established through the research of the past few decades. Protocols have been established for conducting separations of specific classes of compounds and are widely used. This has in a fashion hindered the development of HPLC. Without challenging previous approaches and continuing to rely on past experiences new advances are not common place.

The advent of the commercial chemiluminescent nitrogen detector (CLND) has forced researchers to re-examine the approaches which have been employed for years. The CLND was developed with the pharmaceutical market in mind, providing a detector that can offer universal response to any nitrogen containing compound, regardless of the presence or absence of chromophores. This benefit however comes with a challenge: the eluent cannot contain any nitrogen species.

Pushing this detector beyond the initial vision of those who built it provides a powerful new tool for the analytical chemist, if the separations can be accomplished without nitrogen. The first part of the thesis will deal with the adaptation of current techniques in reverse phase, ion exchange and ion-pair chromatography for use with CLND. The reversed phase separation of cationic and zwitterionic surfactants required the use of barium to block amine-silanol interactions. Ion exchange chromatography illustrated the ability of the CLND to do analysis with a complex matrix under high salt conditions. Ion-pair chromatography of anionic metal-cyanide complexes posed the greatest challenge, requiring the use of a phosphonium cation instead of an ammonium cation as the ion-pairing agent. This led to the

developments explored in the final portion of the thesis: the comparison of ion-pairing characteristics of ammonium, phosphonium and sulfonium cations. The three classes of cation showed significant differences in their selectivities towards several different classes of anions. These differences in retention lead to the beginnings of a potential model on the nature of ion retention in ion pair chromatography.

Acknowledgments

Obviously there are many people whom I wish to thank for their help and support through my time at the University of Alberta. First and foremost among them is Dr. Charles Lucy. Working with you over these years has taught me much about not only analytical chemistry but many facets of both academic and industrial life. I am appreciative of all that I have been able to learn from both your teaching and simply through the example that you set for us.

Of course without my friends the whole of my experience in Edmonton would not be complete. All of those whom I have had the chance to work with have made life here more interesting and more enjoyable. In particular Sarah, Ebbing and Glen have been the best friends and co-workers that anyone could hope for. Your warm friendship will always be remembered with great fondness, I will miss you all very much. There are numerous other members of the department that I would like to acknowledge but the list of good friends would be far too long. I do however want to thank Chris M. and Aaron for the friendship from the first days in the department. As well as Bryan for his help with some of the analysis of the synthetic work. And I could not forget to thank Jayden for his help with the IPA characterization, his skill and dedication were exemplary.

Finally I should thank all those friends and family members who put up with me over the years. Your support and encouragement made the time spent here the most rewarding period of my life. I will miss riding, dancing, climbing and talking with all of you.

TABLE OF CONTENTS

CHAPTER ONE. Introduction

1.1 A New Tool..... 1

1.2 Chemiluminescent Nitrogen Detection..... 2

1.3 Separation Techniques..... 5
  1.3.1 Ion Chromatography..... 6
  1.3.2 Reversed Phase Chromatography..... 8
    1.3.2.1 Bonded Phase Chemistry..... 10
    1.3.2.2 Silanol Activity..... 11
    1.3.2.3 Elution Modes..... 14
  1.3.3 Ion Pair Chromatography..... 16
    1.3.3.1 Ion-Pair Theory..... 18
    1.3.3.2 Double-Layer Theory..... 19
1.4 Thesis Summary..... 22
1.5 References..... 24

CHAPTER TWO. Chemiluminescent Nitrogen Detection in Ion Chromatography for the Determination of Nitrogen Containing Anions.

2.1 Introduction..... 31

2.2 Experimental..... 34
  2.2.1 Chemicals and Materials..... 34
  2.2.2 Sample Preparation..... 34
  2.2.3 Instrumentation..... 35
  2.2.4 Column Cleaning..... 37

2.3 Results and Discussion..... 38
  2.3.1 Equimolar Response..... 38
  2.3.2 Determination of Cyanide..... 45
  2.3.3 Separation and Determination of Other Nitrogen Containing Anions..... 52
  2.3.4 Durability of CLND with Ion Chromatography..... 56

2.4 Conclusions..... 59

2.5 References..... 61

CHAPTER THREE. HPLC Determination of Zwitterionic and Cationic Surfactants Using Chemiluminescent Nitrogen Detection

3.1 Introduction..... 65

3.2 Experimental.......... 68
3.2.1 Chemicals and Materials.......... 68
3.2.2 Sample Preparation.......... 68
3.2.3 Instrumentation.......... 70
3.2.4 Barium Chloride Cleaning.......... 71

3.3 Results and Discussion.......... 73
3.3.1 Column Selection.......... 73
3.3.2 Nitrogen Free Reversed Phase HPLC.......... 74
3.3.3 Cationic Surfactants.......... 79
3.3.4 Zwitterionic Surfactants.......... 82
3.3.5 Linear Range and Limit of Detection.......... 84
3.3.6 Gradient Separation of Zwitterionic Surfactants.......... 86

3.4 Conclusions.......... 89

3.5 References.......... 92

CHAPTER FOUR. Tetrabutylphosphonium Based Ion Pair Chromatography for the Separation of Metal Cyanide Complexes with CLND Detection

4.1 Introduction.......... 95

4.2 Experimental.......... 98
4.2.1 Chemicals.......... 98
4.2.2 Sample Preparation.......... 98
4.2.3 Cleaning of Tetrabutylphosphonium.......... 99
4.2.4 Instrumentation.......... 99

4.3 Results and Discussion.......... 102
4.3.1 Phosphonium/CLND Compatibility.......... 102
4.3.2 Separation of Metal Cyanides with TBP.......... 102
4.3.3 Chromatographic Behaviour.......... 108
4.3.4 Response Factor of Metal Cyanides.......... 110
4.3.5 Limits of Detection.......... 115

4.4 Conclusions.......... 117

4.5 References.......... 119

CHAPTER FIVE. Comparison of Selectivities of Ammonium, Phosphonium and Sulphonium Ion Pairing Agents

5.1 Introduction.......... 122

5.2 Background.......... 125

5.2.1 Hofmeister Series........ 125
5.2.1.1 Anions........ 126
5.2.1.2 Cations........ 127
5.2.2 Collins-Washabaugh Model........ 129
5.2.3 Hofmeister Series and Ion Chromatography........ 132

5.3 Experimental........ 135
5.3.1 Apparatus........ 135
5.3.2 Reagents........ 136
5.3.3 Sulphonium Synthesis........ 137
5.3.4 Eluent Preparation........ 138
5.3.5 Procedure........ 141

5.4 Results and Discussion........ 141
5.4.1 Observed Retention Behaviour with Various IPAs........ 141
5.4.1.1 Kosmotropic Monovalent Anions........ 142
5.4.1.2 Chaotropic Monovalent Anions........ 149
5.4.1.3 Intermediate Monovalent Anions........ 153
5.4.1.4 Multivalent Anions........ 156
5.4.2 Mechanism of IPA Influence on Retention........ 160
5.4.2.1 Influences on Kosmotropic Anions........ 162
5.4.2.2 Influences on Chaotropic Anions........ 163
5.4.2.3 Influences on Intermediate Anions........ 164
5.4.2.4 Influences on Multivalent Anions........ 165
5.4.2.5 Influences of IPA Hofmeister Character on Retention...... 168
5.4.3 Relative Retention........ 169
5.4.3.1 Influence of IPA on Relative Retention........ 169
5.4.3.2 Relative Retention of Chaotropic Anions........ 173
5.4.3.3 Relative Retention of Intermediate Anions........ 174
5.4.3.4 Relative Retention of Multivalent Anions........ 174
5.4.3.5 Overall Impact on Relative Retention........ 175

5.5 Effect of Methanol on Percentage on Anion Retention........ 176
5.5.1 Effect of Methanol on Monovalent Kosmotropic Anions........ 176
5.5.2 Effect of Methanol on Monovalent Chaotropic Anions........ 183
5.5.3 Effect of Methanol on Intermediate Monovalent Anions........ 187
5.5.4 Effect of Methanol on Multivalent Anions........ 191
5.5.5 Overall Effect of Methanol on Ion Pair Chromatography........ 191

5.6 Mode of Influence of Methanol on IPA Separations........ 196
5.6.1 Influence of Methanol on the IPA Equilibrium........ 196
5.6.2 Influence on Water Structure........ 197

5.7 Conclusions........ 199

5.8 References........ 201

CHAPTER SIX. Conclusions and Future Work

6.1 Conclusions.. 208

6.2 Future Work.. 209
    6.2.1 Further Understanding of IPA Separation.............................. 209
    6.2.2 Further Potential for CLND.. 211

6.3 References.. 213

LIST OF TABLES

2.1 Bond dissociations energies of nitrogen, single, double and triple bonds.. 33
2.2 Average maximal signal intensity for replicate (n 5) FIA measurements of 10 mM samples with CLND.. 40
2.3 Comparison of peak areas for replicate (n=3) separations of 50 μM concentrations of nitrite, azide and nitrate with CLND.................... 44
2.4 Comparison of plate heights from the separation of nitrogen containing anions on a Dionex AS 11 column... 51
3.1 Comparison of response factors for the separation of zwitterionic surfactants.. 85
3.2 Composition of CAS U based on peak analysis of the separation......... 91
4.1 Comparison of elution orders of metal cyanide complexes with TBP and TBA.. 104
4.2 Dissociation constant and response factor with CLND for several metal cyanide complexes... 113
4.3 CLND Response factors for metal cyanide complexes..................... 116
5.1 Retention factors for various concentrations of tetrabutylammonium bicarbonate... 143
5.2 Retention factors for various concentrations of tetrabutylphosphonium bicarbonate... 144
5.3 Retention factors for various concentrations of tetrabutylsulphonium bicarbonate... 145
5.4 Retention factors for various percentages of methanol with constant tetrabutylammonium bicarbonate.. 177
5.5 Retention factors for various percentages of methanol with constant tetrabutylphosphonium bicarbonate... 178
5.6 Retention factors for various percentages of methanol with constant tetrabutylsulphonium bicarbonate.. 179

LIST OF FIGURES

1.1 Schematic of the chemiluminescent nitrogen detector.................... 3
2.1 Schematic of ion chromatography system with CLND..................... 36
2.2 Equimolar response for N-containing inorganic anions using CLND.... 39
2.3 Analysis of nitrogen-containing anions in water with IC-CLND......... 43
2.4a Determination of cyanide using IC-CLND and Dionex AS 11 column before column cleaning 46
2.4b Determination of cyanide using IC-CLND and Dionex AS 11 column... 47
2.5 Calibration curve for cyanide with CLND.................................... 49
2.6a Analysis of nitrogen-containing anions in 1:10 seawater by IC with suppressed conductivity detection.. 53
2.6b Analysis of nitrogen-containing anions in 1:10 seawater by IC with UV detection at 214 nm.. 54
2.6c Analysis of nitrogen-containing anions in 1:10 seawater by IC with CLND 55
2.7a Quartz pyrolysis chamber after catastrophic failure during cooling........ 58
2.7b Ceramic only pyrolysis chamber after multiple coolings..................... 58
3.1 Structure of surfactants studied with CLND.................................. 69
3.2 FIA analysis of $BaCl_2$ for nitrogen content with CLND..................... 72
3.3 Isocratic separation of cationic surfactants DTA^+, TTA^+ and HTA^+....... 77
3.4 Gradient separation of triethylamine, benzylamine, heptylamine, dibenzylamine, DTA^+, TTA^+ and HTA^+.. 80
3.5 Isocratic separation of zwitterions Z-1, Z-8, Z-10, Z-12, Z-14 and Z-16... 83
3.6 Linear regression plot for Z-12 surfactant concentrations 50μM-5mM..... 87
3.7 Gradient separation of the industrial zwitterionic surfactant mixture Rewoteric AM CAS U.. 90
4.1 Comparison of CLND response from 100 μM TBP before (a) and after (b) cleaning with anion exchange column.................................. 100
4.2 Separation of metal cyanide complexes with CLND.......................... 106
4.3 Separation of 8 metal cyanide complexes within 15 minutes with CLND.. 109
4.4 Plot of response factors of metal cyanide complexes versus $pK_{dissociation}$... 114
5.1 Schematic of solvated ion based upon the Collins & Washabaugh model involving three layers solvating water... 130
5.2 Retention factors of iodate, chloride and nitrite versus TBA concentration. 146
5.3 Retention factors of iodate, chloride and nitrite versus TBP concentration. 147
5.4 Retention factors of iodate, chloride and nitrite versus TBS concentration. 148
5.5 Retention factors of iodide, thiocyanate and perchlorate versus TBA concentration... 150
5.6 Retention factors of iodide, thiocyanate and perchlorate versus TBP concentration... 151
5.7 Retention factors of iodide, thiocyanate and perchlorate versus TBS concentration... 152
5.8 Retention factors of nitrate and bromide versus TBA concentration......... 154
5.9 Retention factors of nitrate and bromide versus TBP concentration......... 155
5.10 Retention factors of sulphate, chromate and thiosulphate versus TBA concentration... 157

5.11 Retention factors of sulphate, chromate and thiosulphate versus TBP concentration........ 158
5.12 Retention factors of sulphate and thiosulphate versus TBS concentration.. 159
5.13 Relative retention of perchlorate and thiocyanate versus chloride for TBA, TBP and TBS........ 170
5.14 Relative retention of bromide and nitrate versus chloride for TBA and TBP........ 171
5.15 Relative retention of thiosulphate and sulphate versus chloride for TBA, TBP and TBS........ 172
5.16 Log of the retention factors of iodate, chloride and nitrite versus the percent methanol in the TBA eluent........ 180
5.17 Log of the retention factors of iodate, chloride and nitrite versus the percent methanol in the TBP eluent........ 181
5.18 Log of the retention factors of iodate, chloride and nitrite versus the percent methanol in the TBS eluent........ 182
5.19 Log of the retention factors of iodide, thiocyanate and perchlorate versus the percent methanol in the TBA eluent........ 185
5.20 Log of the retention factors of iodide, thiocyanate and perchlorate versus the percent methanol in the TBP eluent........ 186
5.21 Log of the retention factors of iodide, thiocyanate and perchlorate versus the percent methanol in the TBS eluent........ 187
5.22 Log of the retention factors of nitrate and bromide versus the percent methanol in the TBA eluent........ 188
5.23 Log of the retention factors of nitrate and bromide versus the percent methanol in the TBP eluent........ 189
5.24 Log of the retention factors of nitrate and bromide versus the percent methanol in the TBS eluent........ 190
5.25 Log of the retention factors of sulphate, chromate and thiosulphate versus the percent methanol in the TBA eluent........ 192
5.26 Log of the retention factors of sulphate, chromate and thiosulphate versus the percent methanol in the TBP eluent........ 193
5.27 Log of the retention factors of sulphate, chromate and thiosulphate versus the percent methanol in the TBS eluent........ 194

LIST OF SYMBOLS AND ABBREVIATIONS

Symbol	**Parameter**
A	Analyte
A_s	Analyte in stationary phase
A^{x-}	Analyte anion
A^{x-}_m	Analyte anion in mobile phase
C_x	Saturated alkyl carbon chain of x carbon units
CAS U	coco(amindopropyl)hydroxyldimethylsulfobetaine
CLND	Chemiluminescent nitrogen detector(ion)
cmc	Critical micelle concentration
Conc.	Concentration
DTA^+	Dodecyltrimethyl-ammonium
E^{y-}	Eluent anion
EOF	Electroosmotic flow
EPA	Environmental Protection Agency
ESI	Electrospray ionization
eV	Electron volt
FIA	Flow injection analysis
HPLC	High performance liquid chromatography
HTA^+	Hexadecyltrimethylammonium
hv	Photon released from a chemical reaction
i.d.	Inner diameter
IC	Ion chromatography

IHP	Inner Helmholtz plane
IPA(s)	Ion pairing agent(s)
IPC	Ion pair chromatography
k'	Retention factor
L_1	Layer of water closest to the solvated ion
L_2	Second layer of water around a solvated ion
L_3	Third (final) layer of water around a solvated ion
MeOH	Methanol
MS	Mass spectrometry
NMR	Nuclear Magnetic Resonance
o.d.	Outer diameter
OHP	Outer Helmholtz plane
P'	Polarity
P^+	Pairing agent (ion pairing agent)
PEEK	Polyetheretherketone
pH	Negative logarithm of the hydrogen ion concentration
pK_a	Negative logarithm of the dissociation constant
$pK_{dissociation}$	Negative logarithm of the dissociation constant
r	Relative retention
R	Molar gas constant
R^2	Correlation coefficient
RP	Reversed phase
RPLC	Reversed phase liquid chromatography

RSD	Relative standard deviation
T	Temperature
TBA	Tetrabutylammonium
TBP	Tetrabutylphosphonium
TBS	Tetrabutylsulphonium
THF	Tetrahydrofuran
TTA^+	Tetradecyltrimethylammonium
TTAB	Tetradecyltrimethylammonium bromide
UV	Ultra violet
Z-X	Zwittergent® 3-X (X= number of carbons in tail)
ΔH	Enthalpy of retention
$\Delta S°$	Entropy of retention
ϕ	Phase ratio

Chapter 1 Introduction

1.1 A New Tool

The power of an analytical method comes from its ability to both perform a separation as well as to detect the individual components from that separation. Independently a separation or a detection scheme is of limited use analytically. The combination of separation techniques and detectors allows for the identification of a wide range of analytes in various media, including: food products[1-5], blood samples[6-9] and environmental samples[10-12]. The advent of new detector technology opens the door to new potential applications, or the improvement of existing analysis methodologies. However this is not always a seamless operation. The adaptation of new detector technology may require modification of existing separation methodologies, or *vice versa*.

The advent of a commercially available nitrogen specific detector from Antek Industries (Houston, TX, USA) designed for high performance liquid chromatography (HPLC) separations presents a new option for HPLC analysis. The strength of the detector lies in its ability to specifically detect only nitrogen-containing compounds. The detector was intended for pharmaceutical analyses. Yet it proves to be useful in a number of other domains. In the upcoming chapters I will present the adaptation of this chemiluminescent nitrogen detector (CLND) for the determination of both inorganic nitrogen containing anions and surfactants.

The advent of a new detector can however necessitate re-evaluation of the separation process that precedes it. This is particularly true with CLND where the eluent must be absolutely free of nitrogen-containing compounds. Yet this restriction encourages the examination of the practices that have been adopted in HPLC separations, and challenges science to find alternatives. Such is the case that is presented in Chapter 4, detailing ion pair chromatographic separations of metal cyanide complexes are undertaken. The demands imposed by CLND restriction against N-compounds in the eluent altered the way we approached ion pair separations of anions. This change in perspective led to the further exploration of ion pairing agents (IPA) and the first systematic characterization of their separation capabilities (Chapter 5).

1.2 Chemiluminescent Nitrogen Detection

The commercial CLND, Figure 1.1, which was developed by Antek, is based upon the established nitrogen detector for gas chromatography. The adaptation of the gas phase detector for liquid analysis required the ability to transform the aqueous mixture to a gaseous sample. This was accomplished through an ingenious nebulization process that has the effluent of the HPLC flowing through a capillary sheathed in a high volume flow of argon and oxygen gas. The purpose of the sheath gases is two-fold. Firstly, the flow of gas ensures that the sample is nebulized and carried well into the pyrolysis chamber. Secondly, the large volume of oxygen ensures that complete combustion is achieved. To ensure the complete evaporation of the aqueous effluent, the HPLC flow rate is less than 0.3 ml/min. The capillary (77 μm i.d., 153 μm o.d.), does present a limitation as this diameter of capillary, even at the low flow rates of used with the CLND, creates a non-negligible amount of back pressure. This will become a factor when the coupling of ion chromatography and CLND is presented in Chapter 2.

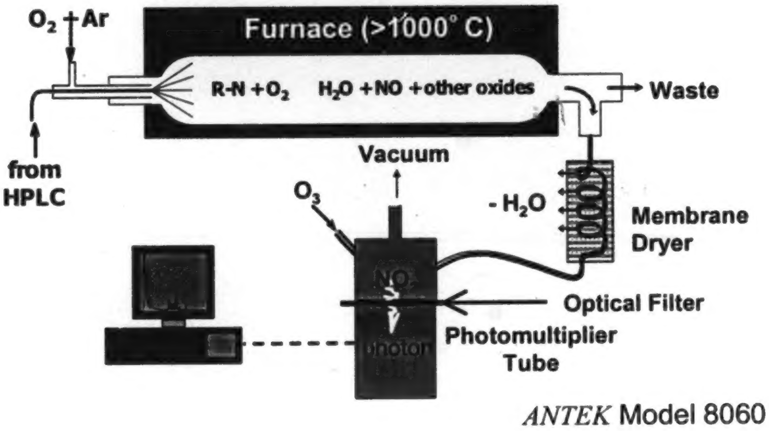

O2 + Ar
Furnace (>1000° C)
R-N + O2 H2O + NO + other oxides
Waste
from HPLC
Vacuum
O3
- H2O
Membrane Dryer
NO2
photon
Optical Filter
Photomultiplier Tube
ANTEK Model 8060

Figure 1.1 Schematic of the Chemiluminescent Nitrogen Detector

Once the sample is nebulized it is combusted at high temperature (>1000°C)[13, 14]:

$$\text{R-N}_{(aq)} + \text{R-H}_{(aq)} + \text{O}_{2(g)} \rightarrow \text{CO}_{2(g)} + \text{H}_2\text{O}_{(g)} + \text{NO}_{(g)} + \text{other products} \qquad (1.1)$$

where R-N is any chemically bound nitrogen compound and R-H is any non-nitrogenous organic compound. The gaseous products of the pyrolysis are drawn by vacuum out of the pyrolysis chamber. From there the gas passes through a length of Nafion® tubing, which is coiled behind one of the CLND ventilating fans. This positioning allows the heated air to pass over the Nafion® tubing drying the gas[15-17], and prevents any condensation from trapping $\text{NO}_{(g)}$. After the dryer, the gas passes through a filter to remove any solids and the gas is then drawn into a reaction chamber with ozone. The subsequent reaction between ozone and nitric oxide (NO) produces nitrogen dioxide in the excited state (NO_2^*):

$$\text{NO}_{(g)} + \text{O}_{3(g)} \rightarrow \text{NO}_2^*{}_{(g)} + \text{O}_{2(g)} + 47.8 \text{ kcal/mol} \qquad (1.2)$$

To ensure the efficient and rapid mixing of the two gases, the inlet lines are joined at approximately 90°, directly in front of the collection aperture of the photomultiplier tube. After reaction the gases are drawn from the chamber by vacuum and carried through an activated carbon filter for cleaning.

The excited nitrogen dioxide formed in reaction 1.2 undergoes a rapid radiative relaxation, resulting in the release of a photon of light between 600 and 900 nm (equation 1.3). This luminescence is then captured and amplified by the photomultiplier tube[13, 14, 18].

$$\text{NO}_2^*{}_{(g)} \rightarrow \text{NO}_{2(g)} + h\nu \qquad (1.3)$$

Other combustion gases such as CO_2 and H_2O do not react with ozone to produce any discernable chemiluminescence, and so do not affect the signal. So long as the ozone in reaction 1.2 is in great excess, the reaction is pseudo first order for NO and the number of photons produced is therefore proportional to the number of NO molecules. Thus, provided the initial combustion step (reaction 1.1) is complete, the chemiluminescent signal will be proportional to the number of nitrogen atoms originally introduced into the detector. To date, only atmospheric diatomic nitrogen has been reported not to produce a chemiluminescence response [13, 14].

The system is ideally designed for use with organic compounds, as both analytes and eluents, where the combustion products are almost entirely gas phase and are quickly removed from the system with no adverse effects. The CLND system has been used for the analysis of drug metabolites[19], the ammonium content of waste waters[20], the purity of synthetic peptides [21], and the classification of pharmaceutical compounds[22]. These separations and most others performed with CLND deal with organic analytes and involve eluents with minimal amounts of inorganic components in the eluent. The focus of my research has been to extend the capabilities of the CLND for the analysis inorganic nitrogen containing species, such as cyanide and metal cyanide compounds.

1.3 Separation Techniques

Modern liquid chromatography encompasses a number of different separation techniques. The main divisions of the separation techniques can be classified as: normal phase, reversed phase, ion exchange and ion pair chromatography. Depending on the requirements of the analysis, different combinations of detector and separation technique will provide the desired information. In my work the detector remained constant, while I adapted various separation techniques to be suitable to its requirements. The following pages outline the key aspects of the separation techniques used in my research.

1.3.1 Ion Chromatography

Modern ion chromatography (IC) has evolved from it's beginnings in 1975 with the work of Hamish Small and co-workers[23]. It is now a widespread and highly accepted as the method for accurate and precise measurements of aqueous inorganic ions and small carboxylic acids and amines. The key to the separation of ions is in the chemistry of the column. The initial ion exchangers were developed solely as ion exchangers, not for high efficiency chromatographic separations. Hence they were designed for high exchange capacity. These exchangers were either silica-based or gel-type exchangers with typical capacities in the range of 0.3-0.5 meq/ml and 0.8-2.4 meq/ml, respectively. However this capacity necessitates high ionic strength eluents in order to elute the ions of interest efficiently. Yet as the work of Gjerde *et al.*[24] showed, if lower capacity ion exchangers were used, then low ionic strength eluents could be used which allows for the detection of ions at the part per billion level using conductivity. Current IC stationary phases have a capacity of 0.006-0.06 meq/ml and are primarily polymeric materials.

The separation of ions from an IC column is based upon an equilibrium between the eluent ions (E^{y-}) and the analyte ions (A^{x-}). For simplicity the remainder of the discussion will focus on anion separations in IC, as this was the work that was undertaken in the course of my research. The anion exchange equilibrium can be represented as:

$$yA^{x-}{}_{m} + xE^{y-}{}_{s} \,\Delta\, yA^{x-}{}_{s} + xE^{y-}{}_{m} \qquad (1.4)$$

The subscripts *s* and *m* denote the anion in the stationary and mobile phases respectively. The equilibrium constant for this exchange will determine the retention of the analyte on the IC column. Different anions will have different equilibrium constants and thus will elute with different retention times.

The retention of the anions can be controlled through the eluent strength and the concentration of the eluent ions (E^{y-}). The most common eluents are, in order of increasing strength: hydroxide; bicarbonate; and carbonate. The strength of an eluent is predominantly related to the charge of the eluent.

The selectivity of an anion exchange separation comes primarily from the column itself, with little impact from the mobile phase. Customization of the stationary phase for a specific sample is common. The primary distributor of IC products, Doinex®, has as many as 18 anion exchange columns commercially available, with the selectivity of the Dionex AS14A tailored for the common anions (F^-, Cl^-, NO_2^-, Br^-, NO_3^-, $H_2PO_4^-$, SO_4^-) and the Dionex AS16 for polarizable anions (I^-, SCN^-, $S_2O_3^-$ and ClO_4^-). Changes in the stationary phase range from alterations in the particle material to changes in the chemistry of the exchange sites. All these changes are designed to alter the equilibrium presented in equation 1.4. Changes in this equilibrium will affect the relative retention of analytes.

The primary detection scheme used for IC is suppressed conductivity. Suppression refers to the reduction of the conductivity of the eluent. For anion exchange chromatography, the suppression is accomplished through the selective exchange of the cations (e.g., Na^+) in the eluent with H^+ using either a cation exchange column or membrane. By exchanging the cations for protons, highly conductive eluent (e.g., OH^-) is converted to a non-conductive species (e.g., H_2O). Simultaneously, suppression enhances the conductivity of strong acid anions (e.g. Cl^-) by replacing the medium conductivity Na^+Cl^- with H^+Cl^-. With suppressed conductivity, detection limits in the low parts-per-billion range are readily achievable for strong acid anions. However, suppressed conductivity is a non-specific detector. Furthermore analytes with a low dissociation constant (e.g., CN^-) experience a decrease in conductivity upon suppression.

UV/visible spectrometry and pulsed amperometric detection can be used for specific analytes. Chapter 2 explores the possibilities of using CLND for detection in ion chromatography.

1.3.2 Reversed Phase Chromatography

Reversed-phase (RP) chromatography is the most common separation method utilized in HPLC. The technique is used for the analysis of a wide range of compounds; including proteins and peptides[25], environmental samples[26], pharmaceutical compounds[27], and biochemicals[28]. This versatility arises from the nature of the retention mechanism, which is based upon the solvatophobic influences. The stationary phase of a RP column is comprised of uniform alkyl chains bound to the stationary phase support. Alkyl chains present a vastly lower polarity than the aqueous mobile phase; water has a polarity (P') of 10.2, methanol of 5.1 and nhexane has a polarity of 0.1[29]. This leads to the partitioning of analytes between the mobile and stationary phases. The equilibrium that is established for an analyte A is:

$$A_m \Delta A_s \quad (1.5)$$

where subscripts m and s refer to the mobile phase and stationary phases, respectively. Equilibrium 1.5 is a function of the polarities of the analyte, the mobile phase and the stationary phase.

The equilibrium of the analyte (Eq. 1.5) is dependant upon the chemical properties of both the analyte and stationary phase. The primary diving force of the equilibrium is the degree of solubility of the analyte in the mobile phase. In RP chromatography the polarity of the mobile phase is significantly higher than that of the stationary phase. The extent of partitioning of the analyte will depend on the energetics required to maintain itself in either environment. As the mobile phase is primarily aqueous, hydrogen bonding will dominate the intermolecular structure. Analytes with greater polarity will require less energy to form a cavity within this hydrogen bonding structure. Conversely an analyte with little polarity will not contribute to the hydrogen bonding and necessitates greater energy to form a cavity within the polar solvent molecules.

The stationary phase however is non-polar, wherein dispersion forces are the primary cohesive influence. This environment favours the adsorption of analytes that are not energetically favoured to be dissolved in the mobile phase. As dispersion forces are significantly weaker than hydrogen bonds the energy required to form a cavity in the stationary phase is far less than that for the mobile phase. Consequently there is the possibility for any analyte molecule to partition into the stationary phase.

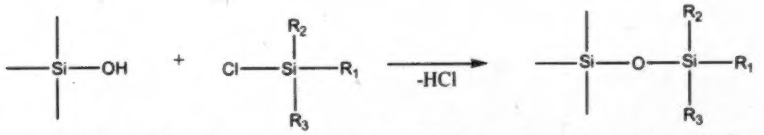
Retention of analytes is controlled through changes to the mobile phase composition, specifically altering the polarity of the mobile phase. This will reduce the energy required to form a cavity in the mobile phase, altering the equilibrium (1.5) and reducing the retention time of analytes. Retention of some analytes can be further altered through changes in eluent pH. Analytes that are acidic or basic can be manipulated to have a charge or not, depending on the eluent pH. The presence of a charge causes the analyte to act as a proton donor or acceptor, increasing its hydrogen bonding ability, decreasing the energy required to form a cavity in the mobile phase.

The stationary phase, as well as the support structure of a column, affects the retention (k') of analytes. For the purpose of this discussion, only silica bonded phases will be explored. The silica bonded phases are by far the most common in RP chromatography[30, 31], and the only type of RPLC stationary phase used in my work. The other support structures that can be used for RP chromatography[32, 33] are primarily polymeric materials[34, 35], zirconia[36] and graphitic carbon[37].

1.3.2.1 Bonded Phase Chemistry

Bonded stationary phases are chemical bound to the silica support structure. Typically highly porous spheres of 5 to 3 μm in diameter are used. Particles as small as 1.0 μm have also been developed[38]. However their use requires specialized pumps and fittings due to the increased backpressure caused by the small particles. Conversely monolithic stationary phases comprised of a continuous (non particular), highly porous silica[39-41] or polymeric[42-44] support are available. Monoliths allow for extremely high flow rates with minimal backpressure. However as the focus of my work is the CLND detector, conventional RP columns were used.

The support structure of traditional RP columns is porous silica. Silica is used due to its structural stability, with particles being able to withstand upwards of 6000 psi while still having substantial porosity. Surface areas for HPLC packings are in the range of 90-600 m^2/g of silica[45]. Silica also offers easy chemical derivatization. A simple derivatization reaction is represented below:



$$-\overset{|}{\underset{|}{Si}}-OH + Cl-\overset{R_2}{\underset{R_3}{Si}}-R_1 \xrightarrow[-HCl]{} -\overset{|}{\underset{|}{Si}}-O-\overset{R_2}{\underset{R_3}{Si}}-R_1 \quad (1.6)$$

R_1 can be either polar (e.g., cyano, amino, diol) to generate a normal phase column, or non polar for reversed phase columns. The most obvious variable between RPLC columns is the alkyl chain length. Retention (k') is directly related to the carbon loading on the column, and this is related to the length of the alkyl chain bonded to the silica. Most commonly, 18 carbon alkyl chains (C_{18}) are used for maximal retention. However, 30, 8, 6, 4 and 1 carbon chain lengths are also available. Additionally normal phase stationary phases such as cyano columns can be used for RPLC, as the linker between the silica and polar functionality provides some RP retention.

1.3.2.2 Silanol Activity

The silica surface beneath the bound stationary phase can also act as a stationary phase. The derivatization reaction (eq. 1.6) does not react with all the silanols (—Si-OH) due to steric hindrances, resulting in less than 4 $\mu mol/m^2$ coverage by the silane in the derivatization process[45]. In comparison silanols are present at 8 $\mu mol/m^2$ in fully hydroxylated silica[45]. Though the exact pK_a of the free silanols is difficult to quantify, in large part due to the influence of impurities, the fact remains that surface silanols are acidic. The values determined for the pK_a of the silanols ranged from values of 4-8, depending on the quality of the silica[46-49]. It appears that the greater the purity of the silica the more acidic the silanols[48]. As a result most modern stationary phases will likely exhibit a negative charge under common eluent conditions. This charge can result in cationic analytes experiencing ion exchange retention, as well as reversed phase retention [48]. The presence of this secondary retention mode can lead to significant deterioration of the quality of a separation due to peak tailing[48, 50].

A number of procedures have been taken by commercial suppliers of RP columns to eliminate this effect. First among these is a procedure known as end-capping. Following the initial derivatization reaction a second derivatization is performed. This time however the functional groups on the silica (R_1, R_2, and R_3) are all methyl groups. This reduces the steric hindrance, allowing the second derivatization to react with residual silanols present between the sites binding the longer alkyl chains. This results in an increased coverage of the silanols on a column improving the separation of basic analytes[47]. The columns used in my research were all end-capped to minimize the problems of silanol interactions.

Alternately, a polar group may be incorporated between the alkyl chain and the silicon. Though this seems counter-intuitive for a reversed phase separation, the improvement in the separation of basic compounds is profound. The polar embedded group decreases the analyte interactions with free surface silanols, significantly improving peak shapes[45, 51, 52]. In addition the nature of the embedded polar group will lead to a change in selectivity[45, 51, 52]. Though this makes the direct incorporation of an existing separation method with a new column challenging, the various selectivities offer the potential for better separations. None of the columns used in my research incorporated the embedded polar group technology.

Another variant on the derivatization process is the incorporation of bulky side chains on the silane used as the linker. Traditionally the side chains R_2 and R_3 (Eq. 1.6) on the silane have been methyls, in order to minimize steric effects and obtain optimal surface coverage in the derivatization. Alternately, the use of isopropyl groups increases the size of the side chains. This extra steric bulk limits ability of analyte molecules to reach the free silanols on the surface[52]. The use of isopropyl groups does not decrease the bonded phase density beyond that seen for other traditional alkyl phases[52]. The most prominent influence of the bulky side chains is the increased stability of the silane bond. This allows for greater stability of the stationary phase, even under conditions of low pH[45, 52]. Though none of the columns used in my research incorporated bulky side chains the Xterra®MS does make use of a variant of this technology. For the Xterra®MS, the silane that is used to bind the stationary phase is tri-functional i.e., it is capable of forming three silane bonds to the surface of the support structure. This helps reduce the number of free silanols present on the surface, while increasing the stability of the chemical bond for the stationary phase[30, 53].

In addition to the derivatization procedures, additives can be included in the eluent to prevent the adsorption of analytes to silanols in the column. Such additives should neither interfere with the primary retention mode nor with detection.

Typically small amines, such as triethylamine are added to the eluent, although alternatives are available[54] as will be discussed further in Chapter 3.

1.3.2.3 Elution Modes

There are two elution procedures utilized in RPLC. The most common elution mode is the use of an isocratic eluent. Isocratic separations use a constant eluent composition throughout the course of the run. The eluent is selected on the basis of its ability to elute all the analytes as quickly as possible while maintaining adequate separation of the analytes. Alternately gradient separations, in which the strength of the eluent is increased over the course of the run, are possible. Each method has its own advantages and disadvantages.

The greatest benefit of isocratic separations is the simplicity, both in the mechanics of the system and of the separation. With a constant eluent the pumping system need not incorporate a mixing system, reducing the cost of the system significantly. The chromatography of isocratic separations is simplified since the conditions of the mobile-phase never change, allowing for a stable equilibrium between the mobile phase and the stationary phase to be established. The throughput of the method is high since there is no down-time due to re-equilibration of the column between runs. The primary disadvantage of the isocratic system arises when analytes possess significantly different retention factors. In order to maintain the separation of all the desired components, careful selection of the eluent must be made. Too strong an eluent will reduce the resolution of the lesser retained species, whereas an eluent that is too weak will lead to poor efficiency and long retention times of the most retained compounds. Finding the ideal balance for such a situation can be challenging, particularly with multiple analytes with significantly different retention factors.

Gradient elution makes use of multiple pumping systems with a mixing chamber[55]. By being able to automatically control the composition of the eluent, the strength of the mobile phase is increased over the course of the run. The benefit of this is that the eluent strength can be tailored to provide the ideal eluent conditions for all compounds of the separation, regardless of their range of retention factors. The result can be ideal resolution and optimal peak shapes for a separation with vast ranges of retention factors of the analytes. The gradient can be controlled to offer a number of different eluent strength profiles, the plot of eluent strength versus time. Most commonly, linear gradients are used, where the eluent strength is increased at a constant rate from a set initial to a set final composition. Other variants include variable rates, exponential rates and the incorporation of plateaus in the eluent strength profile. Each separation will require a unique gradient procedure to optimize the separation depending on the composition of the analytes. The disadvantage of this technique is most evident in the low throughput. Column re-equilibration is necessary upon completion of each run, as the eluent within the column at the end of the run is much stronger than the desired initial conditions. To re-equilibrate the column it is flushed with the eluent strength desired for the initial conditions of the gradient. The equilibration process can take a significant amount of time, drastically reducing the throughput of the method. Additionally if the re-equilibration is not done completely, retention times will be irreproducible[56].

A final factor that can be used as a tool in RP separations is temperature. When controlled properly temperature can be used effectively to reduce retention time, improve separations and improve peak shapes[57-60]. The van't Hoff equation clearly explains the role of temperature in the retention factor (k') of an analyte.

$$\ln k' = -\Delta H/RT + \Delta S°/R + \ln \phi \qquad (1.7)$$

where ΔH and $\Delta S°$ are the respective enthalpy and entropy of the analyte partitioning between the mobile and stationary phase. R is the gas constant and ϕ is the specific phase ratio of the column. It is therefore clear that a change in temperature will alter the retention of an analyte. Depending on the nature of the retention mechanism, this can lead to either an increase or decrease in the retention factor, as has been shown for ion chromatography[61, 62]. For RPLC, changes in temperature will primarily lead to decreases in retention but temperature changes can occasionally yield changes in selectivity[63]. Yet by far the most common use of temperature in RPLC is to ensure reproducibility by preventing temperature fluctuations due to changing laboratory temperature. This is most easily done by using a column heater and setting the temperature several degrees above the hottest likely room temperature[64]. Chapter 2 will provide specific details as to the challenges that were faced when dealing with RP separations, including silanol interactions, isocratic and gradient separations.

1.3.3 Ion-Pair Chromatography

Ion-pair chromatography (IPC) is also frequently referred to as *ion interaction chromatography* or *ion-modified reversed phase chromatography*. For simplicity and consistency the term ion-pair chromatography will be used for all discussions pertaining to this separation method.

The basis of the IPC separation is a hybrid of the ion exchange and reversed phase chromatographic techniques. IPC is designed to provide a separation technique for virtually any ionic species, and in particular to be compatible with highly charged ions (charge >2), which are difficult to separate with IC. In IPC a reversed phase column is used and a hydrophobic ion, such as tetrabutylammonium, is added to the eluent. These ions are referred to as the ion pairing agents (IPA). The IPA will be continuously pumped through the column and will equilibrate between the mobile and stationary phases, as described in Section 1.3.2. This equilibrium will lead to a constant concentration of the IPA partitioned into the stationary phase, resulting in the development of an effective charge within the stationary phase. Due to the presence of this charge, the retention of the analytes will be due to a combination of ionic and hydrophobic interactions.

The eluent composition for an IPC separation is more complex than that used for either IC or RP chromatography. In most cases the eluent is comprised of a set concentration of the IPA, an appropriate organic modifier and a counter-ion/buffer component. All of these components can be varied in both concentration and composition. Though this makes IPC very versatile it also requires a strong understanding of the influences of all aspects to control separations effectively.

There is much debate on the theory of ion retention in IPC. The two views that are most widely held are the "ion-pair theory" and the "double layer theory". Each theory incorporates a different concept of how the analyte ions are retained on the column with respect to the IPA. Though the net results of changes to the eluents will be the same with either model, they take different approaches in understanding how that retention is achieved.

1.3.3.1 Ion-Pair Theory

A number of papers have been published covering the models and variations of the ion-pair theory[65-71]. The following is an overview presenting only those aspects of the theory that will be pertinent to later discussions. Ion-pair theory is based upon the premise that the analyte and the IPA will form a true ion-pair in solution.

$$A^{-}_{m} + P^{+}_{m} \,\Delta\, AP_{m} \,\Delta\, AP_{s} \qquad (1.8)$$

Where A^{-}_{m} is an analyte anion and P^{+}_{m} is the IPA, both in the mobile phase. The two ions can combine to form an ion pair (AP), which can then be adsorbed to the stationary phase; subscripts s and m denote the stationary and mobile phases respectively. This theory takes its roots in the theory of ion-pair extraction, wherein an ion-pair is formed to render the species of interest soluble in a nonpolar solvent. The same concept is applied to the IPC separations, though the partitioning is between the stationary and mobile phases. As stated above, the hydrophobic retention of an ion on a RP column is minimal. Neutralization of the charge of the analyte leads to an increase in retention. Analytes can frequently have little to no native hydrophobicity. Thus the use of a hydrophobic IPA is favoured, as this will increase the retention of all analytes. As such the IPAs used are primarily organic acids for the analysis of cations, and alkyl amines for the analysis of anions. These ions are sufficiently hydrophobic that they experience significant retention on a RP stationary phase, even in their ionic state. This retention of the charged IPA is the basis for a variant of the ion-pair theory; the dynamic ion-exchange model. In this model the IPA is assumed to adsorb to the stationary phase before the analyte forms an ion-pair with it. The energies involved in these two variants of the ion-pair theory can be described by identical retention equations[70]. Regardless of the specific point of view of the retention mechanism, the analyte retention is the result of the adsorption of the associated analyte and IPA.

Alterations of variable components of the eluent are viewed to effect the IPA-analyte associations. The influence of the organic modifier on the retention factor is the same as in RPLC. Increasing the amount of organic modifier (e.g., methanol) decreases the polarity of the mobile phase, which increases the solubility of the associated IPA-analyte, subsequently reducing the retention of the analyte. The IPA concentration is directly related to the analyte retention. Increasing the concentration of the counter ion reduces the analyte retention, by increasing the competition for association by the analyte and the counter ion with the IPA. More counter ions decrease the amount of ion-pairs that are formed with the analyte, decreasing its retention time. Higher IPA concentrations lead to a greater amount of the IPA adsorbed to the stationary phase.-As a result greater amounts of the associated analyte-IPA will be retained, increasing the retention factor. However as the reversed phase column has a finite capacity there is a limitation to the amount of associated IPA that can be adsorbed to the stationary phase, limiting the maximal retention that can be gained through this method.

1.3.3.2 Double-Layer Theory

The double-layer theory views the retention of analytes from a different perspective, one wherein no ion-pairs are formed. The double-layer theory obviously revolves around the formation of a double-layer. The double-layer is developed on the stationary phase through the adsorption of the IPA. Even though the IPA is charged, it has significant hydrophobicity. As the counter ion of the IPA is not significantly hydrophobic only the IPA will be directly adsorbed by the stationary phase. As a consequence of the selective adsorption of only the IPA an accumulation of charge results at the stationary phase. This surface charge leads to the formation of a double-layer, as depicted in Figures 1.2a and 1.2b. As with all double-layers the potential at the stationary phase will decay as the distance from the surface increases. The decay is linear through the compact layer, and subsequently becomes exponential beyond the compact layer, in what is called the *diffuse layer*. At sufficient distance the electrical potential decays to zero in the bulk solution. Unlike the ion-pair theory, the retention of the analytes is not due to direct interaction with the IPA. Instead the analytes, which are opposite to the surface charge, are attracted to the high charge potential at the stationary phase. However the primary retention of the analytes is not viewed as taking place on the stationary phase. The effective stationary phase is taken to be the diffuse layer of the double-layer. This layer originates at the Outer Helmholtz plane (OHP) and is characterized by the exponential decay of the potential originating at the stationary phase. There is a region of solvent between the analyte ions and the IPA ions, which are adsorbed to the stationary phase. Species in the eluent that are capable of covalent or Van der Waals interactions with the IPA will reside closest to the layer of IPAs at the stationary phase, demarked by the inner

Helmholtz plane (IHP)[72]. Ions that are associated to the IPA through interactions other than covalent or Van der Waals forces are limited to a nearest approach distance of the OHP[72]. The retention of the ions comes as result of the electrical potential developed by the adsorbed IPAs. The presence of this field results in a stagnant mobile phase where charged ions are drawn towards the stationary phase, reducing their movement down the column with the mobile phase. The analyte will progress towards the surface until the point where the displacement of solvent molecules between the analyte and the IPA surface becomes greater than displacing those solvent molecules in other directions around the analyte ion[72]. Once the analyte is adsorbed within the diffuse layer the retention mechanism can be expressed as the ion exchange of an analyte and solute ion between the diffuse layer and the bulk solution[73]. Therefore the resulting double-layer that is formed by the adsorbed IPAs can be viewed as developing a dynamic ion exchange surface.

The double-layer theory is simply a theory devised to explain the retention behaviour observed in IPC. Thus, the net result of altering eluent composition is the same as was presented with the ion-pair model (Sec. 1.3.3.1). The difference between the models is the interpretation of why these retention changes occur. As with the ion pair model, increasing the amount of IPA in the eluent will increase retention of the analyte ions until a maximum retention is achieved. At which time a decrease in retention will follow further increases in IPA concentration. The increase in retention is seen as an increase in the electrical potential that is generated at the stationary phase as more IPA ions are adsorbed. This increased potential at the surface leads to higher potentials in the diffuse layer, resulting in a greater stagnant mobile phase within which retention of analytes takes place. Surpassing the capacity of the column to adsorb IPA to the stationary phase will lead to an increase in the ionic strength of the mobile phase, facilitating the suppression of the developed surface potential and reducing the depth of the diffuse layer, decreasing the retention of analytes. Similarly increasing the concentration of counter ions in the eluent will decrease the retention of the analytes. This can be seen as a result of the decrease in the diffuse layer thickness, which is inversely proportional to the ionic strength. Finally the amount of the organic modifier can be altered in the eluent. Increases in the percent methanol will alter the equilibrium established by the IPA between the stationary and mobile phases. Consequently the reduction in concentration of adsorbed IPA will lead to a decrease in the electrical potential of the double-layer, reducing the retention of analytes.

1.4 Thesis Summary

Chapter 2 explores the capabilities of the CLND, beyond the analysis of organic analytes through reversed phased separations. The work examines the analysis of inorganic nitrogen anions through ion chromatography. The application of the CLND system for the analysis of cyanide is of particular interest for the potential for improved limits of detection.

Chapter 3 presents the work that was undertaken on the analysis of cationic and zwitterionic surfactants. The use of the CLND offers a new potential for the analysis of surfactants that are difficult or impossible to analyze through traditional methods. The development of the separation also presents new challenges in the use of additives for HPLC separations.

Examined in Chapter 4 is the ability of the CLND to be utilized as the detector for ion pair chromatographic separations. The analysis of metal cyanide complexes is undertaken with the use of a new ion pairing agent for anion separation. The work compares the use of tetrabutylphosphonium cation to tetrabutylammonium cation for the purpose of the separation of the metal cyanides. The ability of the CLND to quantitatively analyze metal cyanide complexes is also investigated.

The work presented in Chapter 5 will deal with the development of new IPAs for the separation of anions. Through the study of their retention characteristics for a range of anions it will be shown that the above mentioned trends do not hold true for all instances of IPC. In an attempt to further understand the retention process of IPC a new perspective will be presented which will offer a unique view of the mechanism that governs the retention of anions in IPC.

1.5 References

(1) Cohen, R. In *The Simpsons, episode 8f08*; Moore, R., Smart, A., Eds.; 20th Century Fox, 1991.

(2) Belli, N.; Marin, S.; Sanchis, V.; Ramos, A. J. *Food Science and Technology International* **2002**, *8*, 325-335.

(3) Frazier, R. A.; Papadopoulou, A. *Electrophoresis* **2003**, *24*, 4095-4105.

(4) Mello, L. D.; Kubota, L. T. *Food Chemistry* **2002**, *77*, 237-256.

(5) Wardencki, W.; Michulec, M.; Curylo, J. *International Journal of Food Science and Technology* **2004**, *39*, 703-717.

(6) Butler, D.; Guilbault, G. G. *Analytical Letters* **2004**, *37*, 2003-2030.

(7) Cirimele, V.; Villain, M.; Pepin, G.; Ludes, B.; Kintz, R. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences* **2003**, *789*, 107-113.

(8) Jin, O. Y.; Baeyens, W. R. G.; Duan, J. L.; Delanghe, J. *Biomedical Chromatography* **2003**, *17*, 404-410.

(9) Vogeser, M. *Clinical Chemistry and Laboratory Medicine* **2003**, *41*, 117-126.

(10) Halko, R.; Hutta, M. *Chemicke Listy* **2000**, *94*, 990-993.

(11) Hernando, M. D.; Petrovic, M.; Fernandez-Alba, A. R.; Barcelo, D. *Journal of Chromatography A* **2004**, *1046*, 133-140.

(12) Irace-Guigand, S.; Aaron, J. J. *Actualite Chimique* **2004**, 9-14.

(13) Borny, J. F.; Antek Industries: Houston, Texas, 1998.

(14) Yan, X. W. *Journal of Chromatography A* **1999**, *842*, 267-308.

(15) Leckrone, K. J.; Hayes, J. M. *Analytical Chemistry* **1997**, *69*, 911-918.

(16) Sundin, N. G.; Tyson, J. F.; Hanna, C. P.; McIntosh, S. A. *Spectrochimica Acta Part B-Atomic Spectroscopy* **1995**, *50*, 369-375.

(17) Pleil, J. D.; Oliver, K. D.; McClenny, W. A. *Japca-the International Journal of Air Pollution Control and Hazardous Waste Management* **1987**, *37*, 244-248.

(18) Greaves, J. C.; Garvin, D. *Journal of Chemical Physics* **1959**, *30*, 348-349.

(19) Deng, Y. Z.; Wu, J. T.; Zhang, H. W.; Olah, T. V. *Rapid Communications in Mass Spectrometry* **2004**, *18*, 1681-1685.

(20) Fujinari, E. M.; Courthaudon, L. O. *Journal of Chromatography* **1992**, *592*, 209-214.

(21) Fujinari, E. M.; Manes, J. D.; Bizanek, R. *Journal of Chromatography A* **1996**, *743*, 85-89.

(22) Nussbaum, M. A.; Baertschi, S. W.; Jansen, P. J. *Journal of Pharmaceutical and Biomedical Analysis* **2002**, *27*, 983-993.

(23) Small, H.; Stevens, T. S.; Bauman, W. C. *Analytical Chemistry* **1975**, *47*, 1801-1809.

(24) Gjerde, D. T.; Schmuckler, G.; Fritz, J. S. *Journal of Chromatography* **1980**, *187*, 35-45.

(25) Zhu, X. N.; Su, Z. G. *Chinese Journal of Analytical Chemistry* **2004**, *32*, 248-254.

(26) Nelson, M. A.; Gates, A.; Dodlinger, M.; Hage, D. S. *Analytical Chemistry* **2004**, *76*, 805-813.

(27) Wu, N. J.; Dempsey, J.; Yehl, P. M.; Dovletoglou, A.; Ellison, D.; Wyvratt, J. *Analytica Chimica Acta* **2004**, *523*, 149-156.

(28) Tirumalai, R. S.; Chan, K. C.; Prieto, D. A.; Issaq, H. J.; Conrads, T. P.; Veenstra, T. D. *Molecular & Cellular Proteomics* **2003**, *2*, 1096-1103.

(29) McGuffin, V. L. In *Chromatography, 6th ed.*; Heftmann, E., Ed.; Elsevier: Amsterdam, 2004; Vol. 69A.

(30) Claessens, H. A.; van Straten, M. A. *Journal of Chromatography A* **2004**, *1060*, 23-41.

(31) Stella, C.; Rudaz, S.; Veuthey, J. L.; Tchapla, A. *Chromatographia* **2001**, *53*, S113-S131.

(32) Forgacs, E.; Cserhati, T. In *Advances in Chromatography, Vol 40*, 2000; Vol. 40, pp 359-426.

(33) Anderson, D. J. *Analytical Chemistry* **1995**, *67*, R475-R486.

(34) Maikner, J.; Fisher, J.; Gehris, A.; Sherkness, A.; Kinzey, M.; Vanderhof, M. *Lc Gc North America* **2004**, 12-12.

(35) Wang, Q. C.; Svec, F.; Frechet, J. M. J. *Analytical Chemistry* **1993**, *65*, 2243-2248.

(36) Li, J. W.; Reeder, D. H.; McCormick, A. V.; Carr, P. W. *Journal of Chromatography A* **1997**, *791*, 45-52.

(37) Clarot, I.; Cledat, D.; Boulkanz, L.; Assidjo, E.; Chianea, T.; Cardot, P. J. P. *Journal of Chromatographic Science* **2000**, *38*, 38-45.

(38) Patel, K. D.; Jerkovich, A. D.; Link, J. C.; Jorgenson, J. W. *Analytical Chemistry* **2004**, *76*, 5777-5786.

(39) Herrero-Martinez, J. M.; Mendez, A.; Bosch, E.; Roses, M. *Journal of Chromatography A* **2004**, *1060*, 135-145.

(40) Samanidou, V. F.; Ioannou, A. S.; Papadoyannis, I. N. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences* **2004**, *809*, 175-182.

(41) Forlay-Frick, P.; Nagy, Z. B.; Fekete, J. *Journal of Liquid Chromatography & Related Technologies* **2002**, *25*, 1431-1445.

(42) Ping, G.; Schmitt-Kopplin, P.; Hertkorn, N.; Zhang, W. B.; Zhang, Y. K.; Kettrup, A. *Electrophoresis* **2003**, *24*, 958-969.

(43) Mayr, B.; Tessadri, R.; Post, E.; Buchmeiser, M. R. *Analytical Chemistry* **2001**, *73*, 4071-4078.

(44) Buchmeiser, M. R. *Journal of Chromatography A* **2001**, *918*, 233-266.

(45) Neue, U. D. *Encyclopedia of Analytical Chemistry: Applications, Theory and Instrumentation*; Wiley: Chichester, New York, 2000.

(46) Mendez, A.; Bosch, E.; Roses, M.; Neue, U. D. *Journal of Chromatography A* **2003**, *986*, 33-44.

(47) Neue, U. D.; Phoebe, C. H.; Tran, K.; Cheng, Y. F.; Lu, Z. L. *Journal of Chromatography A* **2001**, *925*, 49-67.

(48) Nawrocki, J. *Journal of Chromatography A* **1997**, *779*, 29-71.

(49) Cox, G. B. *Journal of Chromatography A* **1993**, *656*, 353-367.

(50) Stella, C.; Seuret, P.; Rudaz, S.; Carrupt, P. A.; Gauvrit, J. Y.; Lanteri, P.; Veuthey, J. L. *Journal of Separation Science* **2002**, *25*, 1351-1363.

(51) Wilson, N. S.; Gilroy, J.; Dolan, J. W.; Snyder, L. R. *Journal of Chromatography A* **2004**, *1026*, 91-100.

(52) Kirkland, J. J.; Henderson, J. W.; Martosella, J. D.; Bidlingmeyer, B. A.; Vasta-Russell, J.; Adams, J. B. *Lc Gc North America* **1999**, *17*, 634-639.

(53) Li, L.; Carr, P. W.; Evans, J. F. *Journal of Chromatography A* **2000**, *868*, 153-167.

(54) Reta, M.; Carr, P. W. *J. Chrom. A* **1999**, *855*, 121-127.

(55) Dolan, J. W.; Snyder, L. R. In *Encyclopedia of Analytical Chemistry: applications, theory and instrumentation*; Meyers, R. A., Ed.; John Willey and Sons Ltd., 2000; Vol. 13.

(56) Dolan, J. W.; Snyder, L. R. *Troubleshooting LC Systems A Comprehensive Approach to Troubleshooting LC Equipment and Separations*, 1st ed.; Humana Press: Clifton, New Jersey, 1989.

(57) Tran, J. V.; Molander, P.; Greibrokk, Y.; Lundanes, E. *Journal of Separation Science* **2001**, *24*, 930-940.

(58) Guillarme, D.; Heinisch, S.; Rocca, J. L. *Journal of Chromatography A* **2004**, *1052*, 39-51.

(59) Greibrokk, T.; Andersen, T. *Journal of Chromatography A* **2003**, *1000*, 743-755.

(60) Gant, J. R.; Dolan, J. W.; Snyder, L. R. *Journal of Chromatography* **1979**, *185*, 153-177.

(61) Hatsis, P.; Lucy, C. A. *Analyst* **2001**, *126*, 2113-2118.

(62) Hatsis, P.; Lucy, C. A. *Journal of Chromatography A* **2001**, *920*, 3-11.

(63) Dolan, J. W. *Journal of Chromatography A* **2002**, *965*, 195-205.

(64) Wolcott, R. G.; Dolan, J. W.; Snyder, L. R.; Bakalyar, S. R.; Arnold, M. A.; Nichols, J. A. *Journal of Chromatography A* **2000**, *869*, 211-230.

(65) Sarzanini, C.; Bruzzoniti, M. C.; Sacchero, G.; Mentasti, E. *Analytical Chemistry* **1996**, *68*, 4494-4500.

(66) Cecchi, T.; Pucciarelli, F.; Passamonti, P. *Analytical Chemistry* **2001**, *73*, 2632-2639.

(67) Cecchi, T.; Pucciarelli, F.; Passamonti, P. *Chromatographia* **2003**, *58*, 411-419.

(68) Cecchi, T.; Pucciarelli, F.; Passamonti, P. *Journal of Separation Science* **2004**, *27*, 1323-1332.

(69) Bidlingmeyer, B. A.; Deming, S. N.; Price, W. P.; Sachok, B.; Petrusek, M. *Journal of Chromatography* **1979**, *186*, 419-434.

(70) Knox, J. H.; Hartwick, R. A. *Journal of Chromatography* **1981**, *204*, 3-21.

(71) Stranahan, J. J.; Deming, S. N. *Analytical Chemistry* **1982**, *54*, 2251-2256.

(72) Grahame, D. C. *Chemical Reviews* **1947**, *41*, 441-501.

(73) Chen, J. G.; Weber, S. G.; Glavina, L. L.; Cantwell, F. F. *Journal of Chromatography A* **1993**, *656*, 549-576.

Chapter 2 Chemiluminescence Nitrogen Detection in Ion Chromatography for the Determination of Nitrogen Containing Anions[i]

The advent of a chemiluminescence nitrogen detector (CLND) specifically for high performance liquid chromatography opens up a realm of analysis possibilities that were before inaccessible. The detector was designed for use with pharmaceutical products. However the versatility of the instrument allows for it to benefit a number of other areas. This chapter explores the utility of the CLND detector for ion chromatography, specifically focusing on the analysis of inorganic nitrogen-containing anions.

2.1 Introduction

As discussed in Chapter 1 the utility of the CLND comes from both its specificity and universality. Beyond the ability to selectively identify only nitrogen-containing compounds in a complex mixture, the detector provides universal response for all of the nitrogen containing compounds, allowing for easy quantification. To date, all reported determinations with CLND have been for nitrogen-containing organic compounds, all of which produced the expected equimolar response per mole nitrogen. The only nitrogen compound that cannot be detected by CLND is atmospheric diatomic nitrogen[1, 2], due to the strength of the nitrogen triple bond (942 kJ/mol)[3]. This is however highly desirable due to the significant presence of nitrogen gas in the environment. With the current understanding of the chemiluminescent process it is not readily evident that

---

[i] A version of this chapter has been published as Charles A. Lucy and Christopher R. Harrison, *Journal of Chromatography A* **2001**, 920, 135-141.

CLND would be compatible with IC, particularly with inorganic anions. Though it is highly feasible for organic compounds to be volatilized and subsequently combusted in the pyrolysis chamber, it is not apparent that inorganic anions can be effectively transferred into the gas phase. Neither is it apparent that oxygenated anions such as nitrite (NO_2^-) or nitrate (NO_3^-) would be capable of undergoing the combustion or reduction in an oxygen rich environment at elevated temperatures needed to yield NO:

$$NO_x^- \rightarrow NO + (x-1)/2\, O_2 \qquad (2.1)$$

Furthermore the bond strength of many of the anions, such as cyanide, are comparable to that of nitrogen gas[3] (Table 2.1), and would not be expected to undergo the required decomposition. Thus, this chapter investigates the effectiveness of CLND for the ion chromatographic determination of nitrogen-containing inorganic anions including cyanide.

Cyanide is highly toxic and of great concern for both human health[4, 5] and environmental reasons[6-8]. Numerous analysis methods exist for the quantification of cyanide[9-17]. However, most methods for this particular anion are complex. Chromatographic methods are frequently employed for cyanide determination, as they simultaneously provide information on additional compounds present in the sample. In particular, ion chromatography has been employed for the analysis of cyanide in aqueous solutions. However detection is always a challenge for cyanide. Direct UV/visible detection is not possible. Thus post-column reactions are required to enable colorometric[18, 19] or fluorescence

---

Table 2.1 Average bond dissociation energies of nitrogen single, double and triple bonds[3].

Bond Type	Bond Energy (kJ/mol)
N-H	386
N-C	305
N-O	201
N-N	167
N=N	418
N=O	607
N=C	615
N≡C	887
N≡N	942

detection[20, 21]. Alternately either direct or suppressed conductivity can be employed [15, 16, 22].

This study will investigate the robustness of the CLND system when coupled to ion exchange chromatography. As the system was originally designed for primarily salt free or low salt eluents, and not the 0.1- 40 mM salt concentrations used in ion chromatography, the challenges that arise from this operation will be overviewed. In addition, the ability of the detector to handle high salt sample matrices will be investigated with the analysis of several nitrogen containing anions in a synthetic sea water matrix.

2.2 Experimental

2.2.1 Chemicals and materials

All solutions and eluents were prepared in 18 MΩ Nanopure water (Barnstead, Duburque, IO). Sodium hydroxide, sodium nitrite, potassium nitrate and ammonium chloride were from BDH (Toronto, ON). Sodium azide (99% purity) was from Aldrich (Milwaukee, WI). Tetradecyltrimethylammonium bromide (TTAB) (99% pure) and sodium cyanide were from Sigma (St. Louis, MO). Potassium thiocyanate (certified A.C.S.) and oxalic acid (99.9% pure) were obtained from Fisher (Fair Lawn, NJ). Concentrated hydrochloric acid (reagent A.C.S.) was from Anachemia (Montreal, QC).

2.2.2 Sample Preparation

All stock solutions were made to 10 mM concentration in 18 MΩ water, and then diluted to the desired concentration in water, sea water or sodium hydroxide. Stock standards were kept in Nalgene bottles. Samples were prepared fresh daily in 1.5 ml centrifuge tubes. The final concentration of the synthetic sea water[23] was: 40 mM NaCl; 2.5 mM $MgCl_2$; 3 mM Na_2SO_4; 0.75 mM $CaCl_2$; 0.9 mM KCl; 0.25 mM $NaHCO_3$; 20.08 mM KBr (20 mM greater than normal); 0.044 mM H_3BO_3; 0.009 mM $SrCl_2$ and 0.008 mM NaF.

2.2.3 Instrumentation

Figure 2.1 shows a schematic diagram of the ion chromatography instrument used. Ion chromatographic separations with CLND were carried out on a Waters 625 LC system (Waters Associates, Milford, MA). Samples were injected using a Rheodyne 9125 injector (Rheodyne, Berkeley, CA) fit with a 5 or 20 µl injection loop. All connecting tubing was 0.005" i.d. polyetheretherketone (PEEK).

All ion chromatographic separations were performed on a 2 x 250 mm Dionex AS 11 IonPac anion exchange column (Sunnyvale, CA). In flow injection analysis studies, the column was removed such that the injector was connected directly to the detector.

The chemiluminescence detector was an Antek 8060 nitrogen detector (Antek Instruments Inc., Houston, TX). The furnace temperature was set to 1100 °C, the argon flow was 100 ml/min with an oxygen flow of 200 ml/min and the make-up flow comprised of argon at 185 ml/min. The reaction chamber pressure was maintained at 25 torr by the vacuum pump and the ozone flow was 30 ml/min. Flow injection analysis studies were performed with a standard Antek quartz pyrotube, whereas all other analyses were done with a quartz tube containing a ceramic insert to handle the higher salt content of the eluent. Data

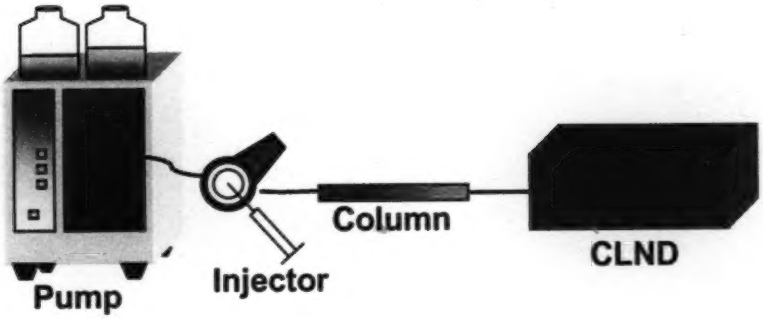

Column
CLND
Pump
Injector

Figure 2.1 Schematic of ion chromatography system with chemiluminescent nitrogen detector.

was acquired at 10 Hz using a National Instrument PC-6023E data acquisition board controlled using Measure software (version 2.0), (National Instruments, Austin, TX) on a 486 microcomputer.

In some separations with the sea water sample the CLND detector was replaced with a Waters 441 Absorbance Detector fit with a Zn lamp to monitor the absorbance at 214 nm. For the suppressed conductivity measurements a Beckman System Gold Model 125 dual piston pump (Beckman, Fullerton, CA) was used to pump eluent at a flow rate of 0.3 ml/min. Injections were performed manually with a Rheodyne 9125 (Rheodyne, Cotati, CA) six-port injection valve fit with a 20 μl loop. Suppressed conductivity detection was measured with a Dionex AMMS-II suppressor and a CDM-3 conductivity detector. A constant pressure pump (< 25 psi) was used to pump 50 mM sulfuric acid at a flow rate of 5 ml/min through the suppressor. Data was collected using a Dionex AI-450 data acquisition system, 10 Hz collection rate, interfaced to a 486 microcomputer.

2.2.4 Column Cleaning

The AS11 column was cleaned based on the manufacturer's suggested procedure[24]. The column was rinsed for 2 hours at 0.6 ml/min with 1 M hydrochloric acid, followed by an hour rinse with 0.1 M oxalic acid and finally 1½ hours with 18 MΩ water. All column washings and rinses were performed in the opposite direction to the standard flow.

2.3 Results and Discussion

2.3.1 Equimolar Response

Previous studies demonstrated that the CLND yields equimolar response for a number of nitrogen-containing organic molecules[25, 26]. That is, amines, amides and nitro- functionalities all yielded a detector response proportional to the number of nitrogen atoms present in the compound, to within ± 15%.

Flow injection analysis studies were conducted with the CLND to test whether such an equimolar response would be achieved for typical inorganic anions of interest in IC. Figure 2.2 shows the results of these experiments. The peak heights are listed in Table 2.2. Ammonium and TTAB were included in the data set as controls. With the exception of azide (N_3^-), the standard deviation of the molar response factor based on peak height is 9.8%. The relative standard deviation of ten replicate injections of 0.5 mM ammonium is 3.1%. Thus, the response for nitrite, nitrate, and cyanide is statistically equivalent to that of amines at the 95% confidence interval.

The response of cyanide is comparable in Figure 2.2 to that of ammonium and TTAB. This was somewhat surprising as the bond dissociation energy for C≡N (887 kJ/mol) is much greater than that for an amine (N-H, 386 kJ/mol) and almost comparable to that of nitrogen gas (N≡N, 942 kJ/mol)[3] to which the CLND does not respond[1, 2]. Though the bond energies appear to be sufficient criteria to select which species will undergo the desired pyrolysis in the CLND it is likely that there are more factors involved. The bond energies do not account for kinetic factors, activation energies and the overall reaction energies that occur. As the

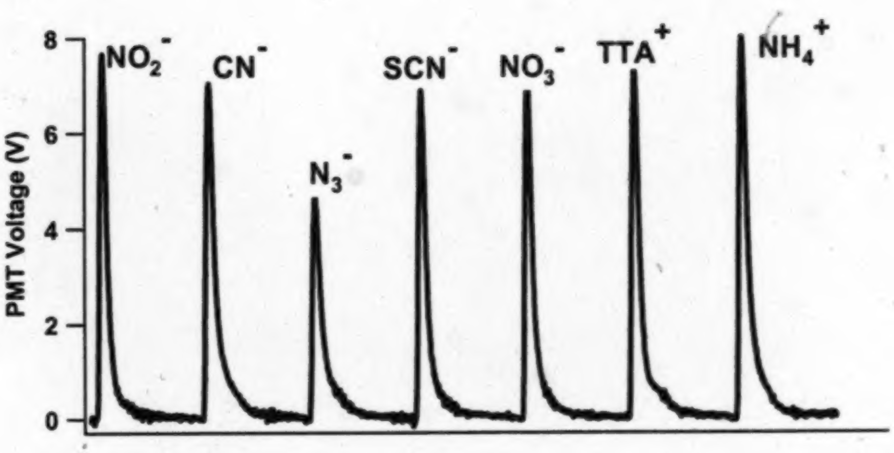


Figure 2.2 Equimolar response for N-containing inorganic anions using CLND. **Experimentation conditions**: flow injection analysis; 5 µl injections of 10 mM of each ion; 0.3 ml/min 18 MΩ water.

Table 2.2 Average maximal signal intensity for replicate (n 5) FIA measurements of 10 mM samples with CLND

Analyte	Average Signal (Volts)	One Standard Deviation
Cyanide	6.09	0.47
Thiocyanate	6.97	0.35
Azide	4.75	0.21
Ammonium	6.79	0.19
Tetradecyltrimethylammonium	7.18	0.21
Nitrite	6.05	0.67
Nitrate	8.02	0.24

pyrolysis is the gas phase reaction of numerous ions the scope of the available literature is limited to the bond energies of the intact analytes. Literature studies do however indicate that under the conditions of the pyrotube cyanide is capable of undergoing the following reaction to yield NO[27].

$$CN + O_2 \rightarrow NCO + O \tag{2.2}$$

$$NCO + O \rightarrow CO + NO \tag{2.3}$$

Once NO has been produced, the remainder of the chemiluminescent reaction, formation of excited NO_2 with ozone, will proceed resulting in a quantifiable signal[2].

In Figure 2.2, nitrite and nitrate also yield a response equivalent to that of ammonium and TTAB. The mechanism by which nitrite and nitrate are decomposed or reduced to NO is unknown. However the results in Figure 2.2 are consistent with previous studies in which signals for nitrite and nitrate were noted but the response factors were not quantified[28]. Sodium nitrite was even used as a standard in the determination of urinary nitrogen[29].

All other nitrogen-containing analytes, which have bond dissociation energies significantly below that of cyanide[3], Table 2.1, would be expected to yield a similar response. Thus, the low response for azide (N_3^-) which contains N=N bonds (418 kJ/mol)[3] was initially somewhat surprising. Azide contains three nitrogen atoms. Other analytes tested by FIA demonstrated responses equivalent to the number of nitrogen atoms per molecule. For instance, caffeine with four nitrogen gave 4.1 times the response of an equal concentration of nitrite. Thus it was expected that 10 mM azide should yield a response three times that of

10 mM of the other N-containing analytes. Yet in Figure 2.2, the response for azide was only 58% that of ammonium. In retrospect the low response for azide is not as surprising, as one of the chief thermal decomposition products of azide is nitrogen gas (N_2)[30].

$$2NaN_3 \rightarrow 2\,Na + 3N_2 \tag{2.4}$$

Certainly it is because of its rapid decomposition to N_2 that sodium azide is used in emergency air bags in automobiles[31]. The pyro-chemiluminescence detector does not respond to nitrogen gas[1, 2]. Nevertheless, some response was observed for azide. Studies of the thermal decomposition of azide containing compounds (*i.e.*, explosives) such as poly(bis(3,3'-azidomethyl)oxetane) (-($C_5H_8N_6O$)-) and poly(glycidylazide) (-$C_3H_5N_3O$-) indicate that 31.7 to 37.4% of the nitrogen is converted to ammonia, hydrogen cyanide and/or other nitro-products [32], to which the CLND would respond. However this does not coincide with the response that was seen for 10 mM azide (Figure 2.2). If one third of the nitrogens formed detectable species (non nitrogen gas) the signal from azide should be approximately equal to that of the single nitrogen containing species of the same concentration. However it is evident in Figure 2.2 that the response for azide is less than that of the other ions, despite the fact that they are equimolar. This relative response appears to depend upon the concentration of azide being analyzed. When the concentration of azide is reduced from 10 mM to 50 μM (Figure 2.3) the response is equivalent to that of 50 μM nitrate. The peak areas are listed in Table 2.3, and it can be seen that the percent difference in peak area is less than 3%, versus that of nitrate. This indicates that the formation of N_2

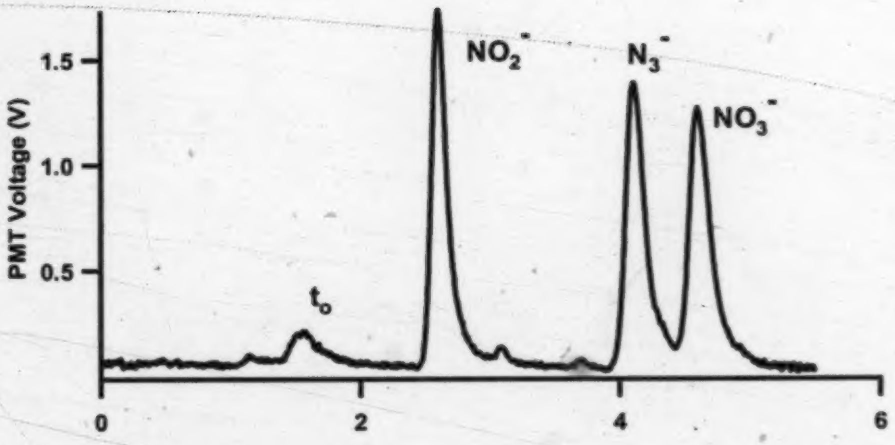


Figure 2.3 Analysis of nitrogen-containing anions in water with IC-CLND

Experimental conditions: Dionex AS11 column; 5 mM NaOH at 0.3 ml/min; 20 μl injection of 50 μM each of NO_2^-, N_3^- and NO_3^-.

Table 2.3 Comparison of peak areas for replicate (n=3) separations of 50 μM concentrations of nitrite, azide and nitrate (conditions as in Fig. 2.3) with CLND.

	Average Peak Area	**One Standard Deviation**	**Percent Difference (compared to Nitrate)**
Nitrite	148.7	6.6	0.4 %
Azide	145.5	9.5	2.5 %
Nitrate	149.3	7.2	—

through combustion of azide is dependant upon the amount of azide present. It appears that azide compounds will yield approximately 33% percent of the nitrogen in forms other than N_2, only if the concentration analyzed is small enough. The dead time marker in this separation and all others was a nitrogen impurity, non-ionic or cationic, which was found to elute at the dead time.

2.3.2 Determination of Cyanide

Studies were performed to determine the sensitivity of the CLND detector for cyanide. Hydrogen cyanide is a relatively weak acid (pKa = 9.22). Thus, under the acidic eluent conditions (pH < 7)[33] which exist after suppression, cyanide is largely protonated. As a consequence, the limit of detection for cyanide with suppressed conductivity is much lower than for other anions. Recently, Caliamanis *et al.*[22] demonstrated that the detection limits for cyanide using suppressed conductivity could be improved to 50 μM by using a second anion micromembrane suppressor to convert HCN into NaCN, much in the manner of Sjogren and Dasgupta[34].

The separation conditions of Caliamanis *et al.*[22] (Dionex AS11 column and 5 mM NaOH as eluent) were used herein. Initially an extremely tailed peak was observed as is shown in Figure 2.4a. The measured efficiency of this peak (N = 313 based on the tangent method) was comparable to that of Caliamanis *et al.*, but much poorer than the 5000 plates achieved for other anions. Further, the elution behavior of cyanide was irreproducible. Cleaning of the column as described in Section 2.2.4 yielded much more efficient peaks (N=1200) for

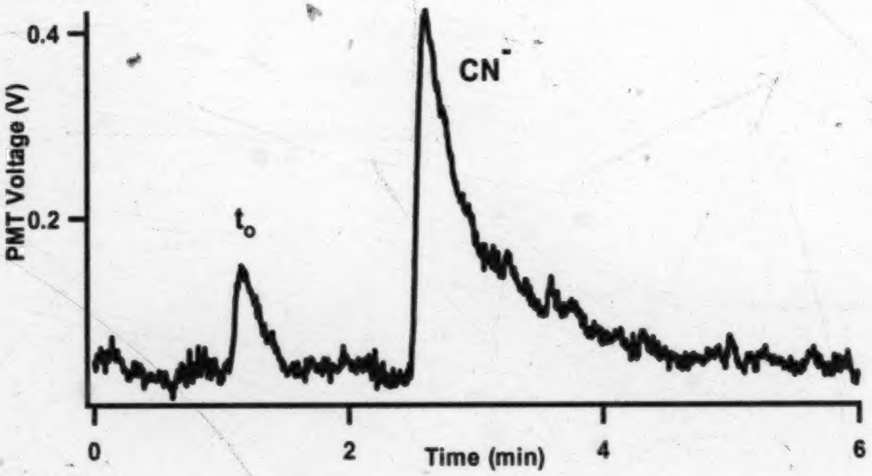


Figure 2.4a Determination of cyanide using IC-CLND and Dionex AS11 column before column cleaning. **Experimental conditions:** eluent: 5 mM NaOH at 0.3 ml/min; 20 µl injection of 50 µM cyanide.

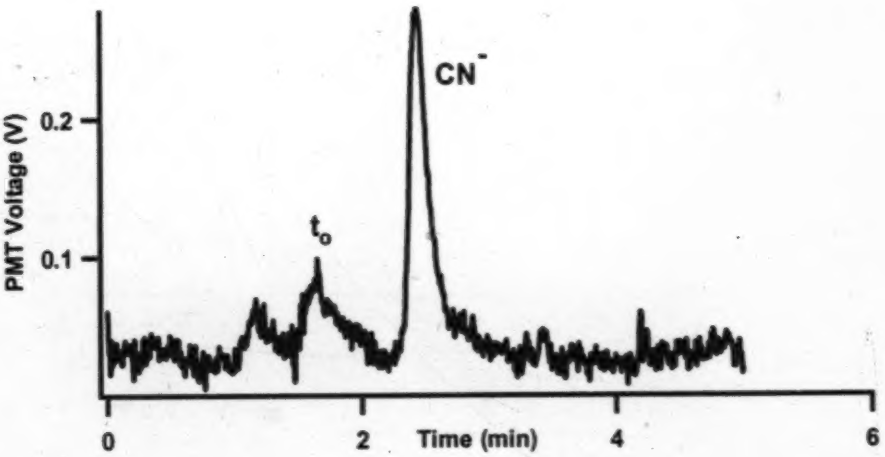


Figure 2.4b Determination of cyanide using IC-CLND and Dionex AS11 column. **Experimental conditions:** Column after cleaning procedure (Section 2.4); eluent: 5 mM NaOH at 0.3 ml/min; 20 μl injection of 10 μM cyanide.

cyanide, as shown in Figure 2.4b. Although we did not identity the contaminants on the column, it is reasonable to suspect they are metal species. Metals, particularly iron, are capable of forming relatively stable complexes with cyanide under the eluent conditions[35, 36]. Studies have shown that the presence of metal impurities in HPLC columns can lead to significant peak distortion, particularly peak tailing[37, 38]. Though the system used for this study was entirely PEEK the column had been used previously with a stainless steel system. Engelhardt *et al.*[38] showed that the use of a stainless steel system can rapidly lead to the accumulation of significant amounts of metal impurities on a column. For all future work with cyanide analysis it is highly recommended to use metal free systems as much as possible.

Calibration curves (Figure 2.5) based on peak area were linear (R=0.998) over the range studied (5-100 μM) with an intercept statistically equal to zero. The precision measured for 7 injections at 25 μM was 9% RSD. The detection limit for cyanide at the 95% confidence interval was 3 μM (78 μg/l), as determined using the EPA procedure[39]. This method is performed with a sample that is one to five times greater than the estimated limit of detection. This sample is to be analyzed 7 or more times, as well as an equal number of blank samples. The average blank value is subtracted from the value of each of the samples. The standard deviation of the sample measurements is then multiplied by the appropriate Student's *t* value for the desired percent confidence. The concentration detection limit of 3 μM corresponds to a mass detection limit of 0.8 ng-N, which is close to the 0.1 ng-N typically quoted for CLND[1]. The

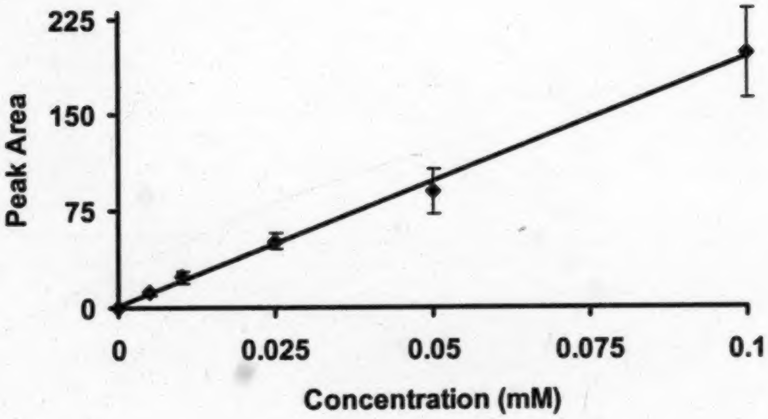


Figure 2.5 Calibration curve for cyanide with CLND. Error bars represent one standard deviation.

concentration detection limit using CLND is more than an order of magnitude lower than that recently reported for suppressed conductivity with an ion exchange reactor[22], and comparable to that achievable using direct conductivity detection[33] or suppressed conductivity after oxidation of cyanide to cyanate[15]. However, detection limits achieved with derivatization[20, 40] and amperometry[41] are significantly lower, albeit the latter is prone to interference by chloride[42]. Though this work demonstrates a linear response over an order of magnitude, Chapter 3 (Sec. 3.3.5) illustrates the large dynamic range of the CLND.

Using the AS11 column, cyanide was separated from other nitrogen containing anions such as azide and nitrate, but co-eluted with nitrite. Attempts were made to allow for the separation of the nitrate and cyanide. Chief among these was the use of an alternate anion separation column, the Dionex AS14, with both a carbonate/bicarbonate and a hydroxide eluent. Separations were tested over a range of eluent concentrations, 0.5-1.6 mM carbonate and 0.12-0.30 mM bicarbonate. No significant changes in selectivity were observed. The use of a different column with an appropriate eluent should be able to separate cyanide from all other nitrogen containing anions. Ideally Virtual Column 2[43], a software program for the simulation of IC separations, available for download at http://www.virtualcolumn.com could be used for predictions. However the software does not include cyanide as an analyte at this time.

Table 2.4 Comparison of plate heights from the separation of nitrogen containing anions on a Dionex AS11 column (conditions as in Fig. 2.3).

	Plate Height
Cyanide	1200
Nitrite	2872
Azide	4603
Nitrate	4021

2.3.3 Separation and Determination of Other Nitrogen Containing Anions

Separations of nitrite, azide and nitrate were achieved using the AS11 column with a 5 mM sodium hydroxide eluent. A typical chromatogram is shown in Figure 2.3. The efficiency was calculated for the three anions and is tabulated with that of cyanide in Table 2.4. The efficiency obtained for the anions was greater than that of cyanide and approaching the expected 5000 plates for an anion separation. Detection limits for these anions in 18 MΩ water was about 3 μM (0.8 ng-N), which was comparable to that achieved for cyanide above. However one of the CLND detector's advantages is that it is insensitive to non-nitrogen containing species. Thus it should be ideal for determination of nitrite, azide and nitrate in complex matrices. Figure 2.6 shows chromatograms for 50 μM of nitrite, azide and nitrate in a 1:10 dilution of synthetic sea water (Section 2.2.2). Azide and nitrate are detectable at this level using suppressed conductivity (Figure 2.6a). Some overlap with the bromide peak is apparent. However the level of Br^- in the synthetic sea water (20 mM) is significantly higher than would normally be observed, and so this overlap would not normally cause a problem. The nitrite peak however is completely lost in the tail of the large chloride signal.

Direct UV absorbance is often used for detection of nitrite and nitrate[44]. Figure 2.6b shows the chromatogram observed when UV detection at 214 nm was used to determine these anions in 1:10 diluted sea water. Some slight shift in retention times is evident due to differences in the length of tubing connecting the column to the various detectors. More importantly however is that this

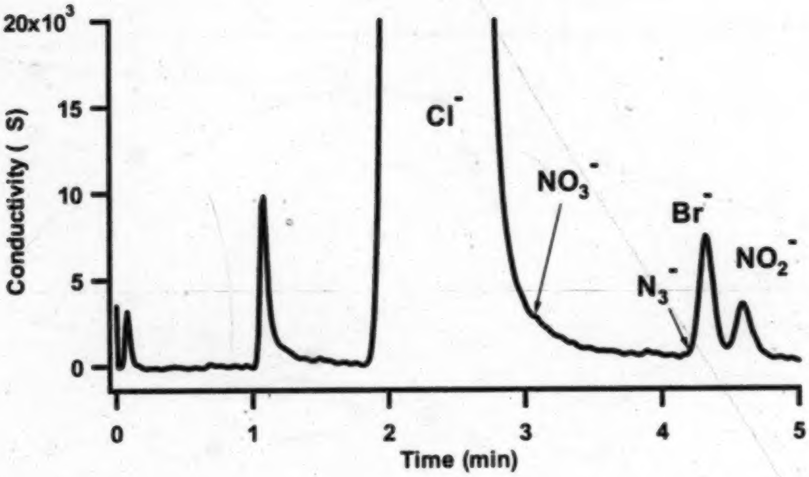


Figure 2.6a Analysis of nitrogen-containing anions in 1:10 seawater by IC with suppressed conductivity detection. Experimental conditions as in Figure 2.4

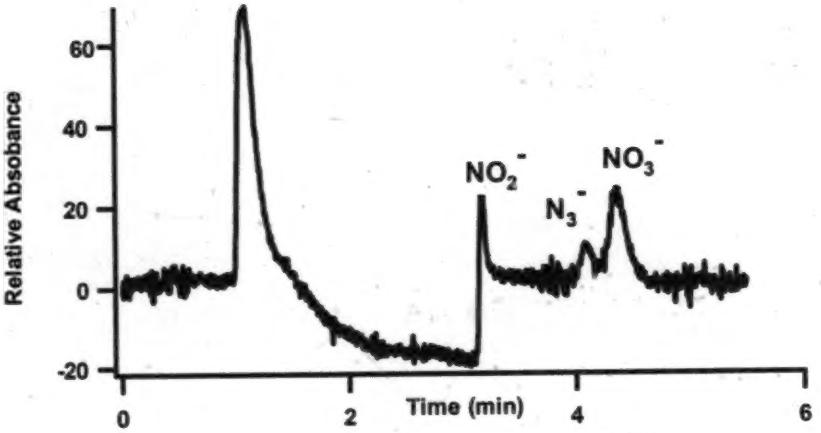


Figure 2.6b Analysis of nitrogen-containing anions in 1:10 seawater by IC with UV absorbance detection at 214 nm. Experimental conditions as in Figure 2.4

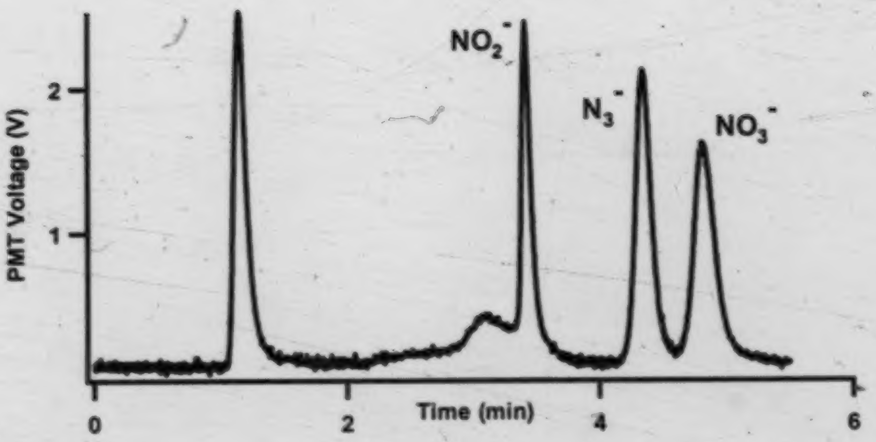


Figure 2.6c Analysis of nitrogen-containing anions in 1:10 seawater by IC with CLND. Experimental conditions as in Figure 2.4

chromatogram is far from appealing. The chloride causes a large negative peak, caused by the displacement of the absorbance background created by the hydroxide. This negative peak almost completely obscures the nitrite peak. The azide and nitrate peaks are still visible but disproportionate due to their differing absorptivities and are potentially masked by other UV absorbing anions such as bromide.

In contrast, CLND yields a chromatogram (Figure 2.6c) that is almost indistinguishable from that in 18 MΩ water (Figure 2.3). The chloride matrix has caused a slight shift to longer retention times, with some changes to the peak efficiencies as compared to the simpler matrix (Table 2.3). The origin of the peak just prior to nitrite is unknown. However, it does not appear interfere with the determination of nitrite.

2.3.4 Durability of CLND with Ion Chromatography

Initially one would not conceive of ion chromatography as being compatible with a gas phase analyzer such as the CLND. Particularly the eluent would be expected to cause severe problems due to the salt content. Yet the chemistry of the pyrolysis chamber is in fact amenable to the eluents used for chromatographic separations of anions. The high temperature and oxygen content of the pyrolysis chamber favours the elimination of either carbonate or hydroxide as carbon dioxide or water. A potential reaction scheme for the decomposition of sodium bicarbonate and sodium carbonate are listed below:

$$2NaHCO_{3(aq)} \rightarrow H_2O_{(g)} + 2CO_{2(g)} + Na_2O_{(s)} \qquad (2.5)$$

$$Na_2CO_{3(aq)} \rightarrow Na_2O_{(s)} + CO_{2(g)} \qquad (2.6)$$

The only residue that would be left behind would be the sodium ion, likely converted to the oxide. However, if a suppressor were incorporated into the system, the sodium cation would have been exchanged for H^+ before reaching the CLND. Thus no deposition would result from the use of the carbonate/bicarbonate eluent. Unfortunately, at the time that this work was performed the commercially available suppressors for ion chromatography were incompatible with the CLND. In particular the suppressors were of a membrane style which could not withstand the backpressure (typically 200 psi) created by the CLND nebulizer. However current suppressors such as the Metrohm MSM and Dionex Atlas® suppressors are much more robust and capable of withstanding much higher back pressures than membrane suppressors[45]. Thus suppressors could be used to convert the sodium in the eluent to hydrogen, providing a perfectly clean eluent for the CLND.

In the absence of post-separation suppression, there were a number of limitations in the long-term use of CLND with ion chromatography. In particular the amount of salt deposited within the pyrotube became an issue. The manufacturer's literature states that the pure quartz pyrotube can be used with eluents composed of up to 25 mM salt [30]. However, after only 2 weeks of use with a 5 mM sodium carbonate/bicarbonate buffer, our quartz pyrotube shattered upon cooling (Figure 2.7a) from 1050 °C to room temperature. The use of a pyrotube with a ceramic insert, Figure 2.7b, provided an increase in lifetime. Nonetheless, after approximately one month of use, the quartz portion of the quartz/ceramic pyrotube shattered upon cooling. The most recent pyrotube developed by Antek is made entirely of ceramic. Over the course of two years of

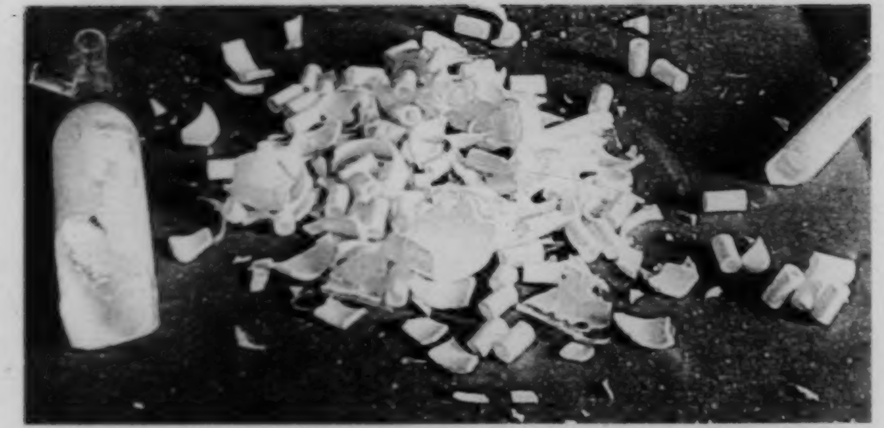


Figure 2.7a Quartz pyrolysis chamber after catastrophic failure during cooling

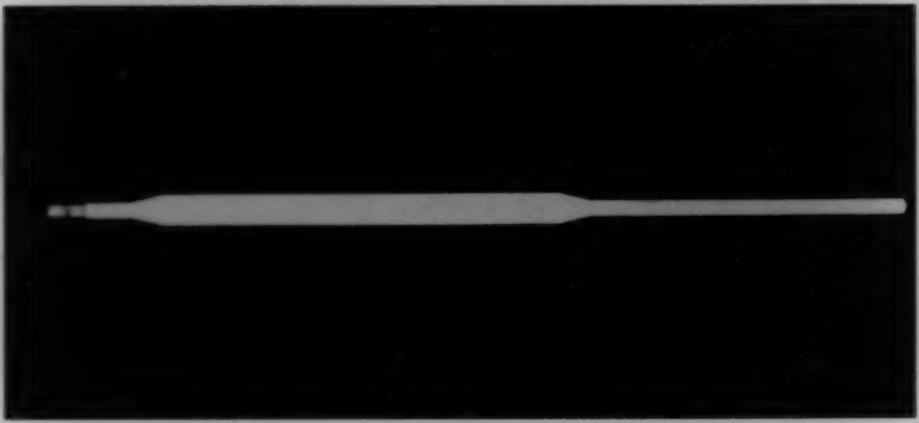


Figure 2.7b Ceramic only pyrolysis chamber after multiple coolings

use and 3 heating and cooling cycles, this pyrotube has remained intact, even with continuous use with moderate concentrations of non-volatile eluents.

The ceramic pyrotube has eliminated the most catastrophic problem with long-term use of the CLND. However, the use of a suppressor is still desirable. It would extend the life of any quartz-based pyrotube and reduce the need for cleaning of the ceramic pyrotubes. Also more concentrated eluents could be used without detector fouling becoming an issue. Further, there were a number of other consequences to long-term exposure to high salt content eluents. The majority of the additional problems arose from salts traveling beyond the pyrotube. This led to crystallization of salts within the outlet port of the pyrotube (Figure 1.1) severely limiting gas (analyte) flow to the ozonolysis chamber. The salts also contaminated the Nafion™ dryer tube (Figure 1.1), causing it to dry and crack. Finally salt eventually passed through the filter and contaminated the ozonolysis chamber where deposition of salt on the photomultiplier tube window reduced sensitivity. The majority of these problems would be minimized with the use of a suppressor. Regardless, it remains critical to choose eluent additives that vaporize or combust under the pyrolysis conditions.

2.4 Conclusions

The chemiluminescence nitrogen detector (CLND) yields low micromolar concentration detection limits and sub-ng-N mass detection limits for nitrogen-containing anions such as nitrite, nitrate, azide and cyanide. With the exception of azide, the response of these anions is proportional to the amount of nitrogen in the anions. Their relative standard deviation for the molar response was less than

10%. Within that precision range the CLND is capable of universal detection of nitrogen containing compounds. The selectively of the detector to nitrogen containing species makes it extremely effective in analysis of complex matrices such as sea water.

2.5 References

(1) Borny, J. F.; Antek Industries: Houston, Texas, 1998.

(2) Yan, X. W. *Journal of Chromatography A* **1999**, *842*, 267-308.

(3) Chang, R. In *Chemistry, 4th edition*; McGraw-Hill: New York, 1991, pp 377.

(4) Lindsay, A. E.; Greenbaum, A. R.; O'Hare, D. *Analytica Chimica Acta* **2004**, *511*, 185-195.

(5) Houeto, P.; Borron, S. W.; Marliere, F.; Baud, F. J.; Levillain, P. *Indoor and Built Environment* **2001**, *10*, 62-69.

(6) Barnes, D. E.; Wright, P. J.; Graham, S. M.; Jones-Watson, E. A. *Geostandards Newsletter-the Journal of Geostandards and Geoanalysis* **2000**, *24*, 183-195.

(7) Haghighi-Podeh, M. R.; Siyahati-Ardakani, G. *Water Science and Technology* **2000**, *42*, 125-129.

(8) Boening, D. W.; Chew, C. M. *Water Air and Soil Pollution* **1999**, *109*, 67-79.

(9) Jin, W. J.; Costa-Fernandez, J. M.; Pereiro, R.; Sanz-Medel, A. *Analytica Chimica Acta* **2004**, *522*, 1-8.

(10) Zheng, A. P.; Dzombak, D. A.; Luthy, R. G.; Sawyer, B.; Lazouskas, W.; Tata, P.; Delaney, M. F.; Zilitinkevitch, L.; Sebroski, J. R.; Swartling, R. S.; Drop, S. M.; Flaherty, J. M. *Environmental Science & Technology* **2003**, *37*, 107-115.

(11) Sheu, S. H.; Weng, H. S. *International Journal of Environmental Analytical Chemistry* **2000**, *78*, 107-115.

(12) Volmer, W.; Giesselmann, G. *American Laboratory* **2000**, *32*, 18-+.

(13) Sequeira, M.; Hibbert, D. B.; Alexander, P. W. *Electroanalysis* **1999**, *11*, 494-498.

(14) Carr, S. A.; Baird, R. B.; Lin, B. T. *Water Research* **1997**, *31*, 1543-1548.

(15) Nonomura, M. *Analytical Chemistry* **1987**, *59*, 2073-2076.

(16) Otu, E. O.; Byerley, J. J.; Robinson, C. W. *International Journal of Environmental Analytical Chemistry* **1996**, *63*, 81-90.

(17) Say, R.; Ersoz, A.; Turk, H.; Denizli, A. *Separation and Purification Technology* **2004**, *40*, 9-14.

(18) Inoue, Y.; Suzuki, Y.; Ando, M. *Bunseki Kagaku* **1993**, *42*, 617-623.

(19) Sun, B. T.; Noller, B. N. *Water Research* **1998**, *32*, 3698-3704.

(20) Gamoh, K.; Imamichi, S. *Analytica Chimica Acta* **1991**, *251*, 255-259.

(21) Sumiyoshi, K.; Yagi, T.; Nakamura, H. *Journal of Chromatography A* **1995**, *690*, 77-82.

(22) Caliamanis, A.; McCormick, M. J.; Carpenter, P. D. *Journal of Chromatography A* **2000**, *884*, 75-80.

(23) Lyman, J.; R.H., F. *Journal of Materials Research* **1940**, *3*, 134.

(24) Corporation, D.; Dionex Corporation, 2000; Vol. 2000.

(25) Taylor, E. W.; Qian, M. G.; Dollinger, G. D. *Analytical Chemistry* **1998**, *70*, 3339-3347.

(26) Fujinari, E. M.; Manes, J. D.; Bizanek, R. *Journal of Chromatography A* **1996**, *743*, 85-89.

(27) Glarborg, P.; Kristensen, P. G.; Jensen, S. H.; Damjohansen, K. *Combustion and Flame* **1994**, *98*, 241-258.

(28) Fujinari, E. M.; Courthaudon, L. O. *Journal of Chromatography* **1992**, *592*, 209-214.

(29) Boehm, K. A.; Ross, P. F. *Journal of Aoac International* **1995**, *78*, 301-306.

(30) Dyke, J. M.; Groves, A. P.; Morris, A.; Ogden, J. S.; Catarino, M. I.; Dias, A. A.; Oliveira, A. M. S.; Costa, M. L.; Barros, M. T.; Cabral, M. H.; Moutinho, A. M. C. *Journal of Physical Chemistry A* **1999**, *103*, 8239-8245.

(31) Madlung, A. *Journal of Chemical Education* **1996**, *73*, 347-348.

(32) Roos, B. D.; Brill, T. B. *Applied Spectroscopy* **2000**, *54*, 1019-1026.

(33) Okada, T.; Kuwamoto, T. *Analytical Chemistry* **1985**, *57*, 258-262.

(34) Sjogren, A.; Dasgupta, P. K. *Analytical Chemistry* **1995**, *67*, 2110-2118.

(35) Ghosh, R. S.; Dzombak, D. A.; Luthy, R. G. *Environmental Engineering Science* **1999**, *16*, 293-313.

(36) Meeussen, J. C. L.; Keizer, M. G.; Dehaan, F. A. M. *Environmental Science & Technology* **1992**, *26*, 511-516.

(37) Slingsby, R. W.; Bordunov, A.; Grimes, M. *Journal of Chromatography A* **2001**, *913*, 159-163.

(38) Engelhardt, H.; Lobert, T. *Analytical Chemistry* **1999**, *71*, 1885-1892.

(39) Harris, D. C. In *Quantitative Chemical Analysis*; W.H. Freeman and Company: New York, 1996; Vol. 4th Edition, pp 84.

(40) Madungwe, L.; Zaranyika, M. F.; Gurira, R. C. *Analytica Chimica Acta* **1991**, *251*, 109-114.

(41) Jandik, P.; Cox, D.; Wong, D. *American Laboratory* **1986**, *18*, 114-&.

(42) Seneviratne, J.; Holmstrom, S. D.; Cox, J. A. *Talanta* **2000**, *52*, 1025-1031.

(43) Haddad, P. R.; Shaw, M. J.; Madden, J. E.; Dicinoski, G. W. *Journal of Chemical Education* **2004**, *81*, 1293-1298.

(44) Romano, J. P.; Krol, J. *Journal of Chromatography* **1992**, *602*, 205-211.

(45) Haddad, P. R.; Jackson, P. E.; Shaw, M. J. *Journal of Chromatography A* **2003**, *1000*, 725-742.

Chapter 3 HPLC Determination of Zwitterionic and Cationic Surfactants Using Chemiluminescent Nitrogen Detection[1]

This chapter examines the combination of the chemiluminescent nitrogen specific detector (CLND) with an HPLC separation that allows for the identification and quantification of cationic and zwitterionic surfactants. The fact that the CLND provides equimolar responses, based on the amount of nitrogen, allows for easy quantification of the nitrogen containing components of a surfactant mixture. HPLC-CLND separations will be used for the determination of an industrial zwitterionic surfactant, coco(amidopropyl)hydroxyldimethylsulfobetaine (Rewoteric AM CAS U). A cyano column was used to develop the separation of cationic and zwitterionic (sulfobetaine) surfactant mixtures. The limits of detection for these surfactants are in the single-digit micromolar range (1 ng N). A linear response ($R^2 = 0.9981$) was obtained for a zwitterionic surfactant between 50 μM and 5 mM.

3.1 Introduction

Surfactants are a class of compounds that are found in a multitude of domains, from industrial settings, to research labs, to household products as well as environmental pollutants. Due to their prevalence in so many domains the need for analysis is paramount [1]. Ionic surfactants can be classified into three distinct categories depending on the charge of their hydrophilic portion: cationic, anionic and zwitterionic (amphoteric). The majority of the analysis of surfactants present in the literature pertains to the analysis of anionic surfactants. This chapter focuses on cationic and zwitterionic surfactants, for which very few analysis procedures exist [2-4].

---

[1] A version of this chapter has been published as Christopher R. Harrison and Charles A. Lucy, *Journal of Chromatography A* **2002**, 956, 237-244.

Of the methods available for the analysis of cationic or zwitterionic surfactants, most are developed for, and restricted to, a specific class of cationic or zwitterionic surfactant. This is in large part due to the difficulties in analysis of different surfactant species. Direct UV analysis is possible for a few of the surfactants; generally it is restricted to benzyl or pyridyl containing surfactants[5]. For those compounds that are not UV active, indirect detection is a possibility[6]. However this requires the proper combination of UV chromophore and analyte to be effective. Additionally the sensitivity of indirect detection is limited[7]. Alternate detection schemes suffer from additional restrictions. These arise either from the separation conditions or more commonly from the specific post-column reaction detection methods. This can be illustrated by the detection scheme used for imidazoline type surfactants used by Kawase *et al.* [8]. In the work by Kawase *et al.* the detection scheme was based on the direct and indirect UV identification of the reaction products of the imidazoline surfactants after they were reacted with sodium hydroxide and/or sodium chloroacetate. Though highly effective for the analysis of imidazoline type surfactants the detection scheme cannot be applied to other classes of surfactants. Other more common post-column reactions rely on the formation of ion-pairs which then need to be extracted from the eluent stream prior to being detected spectroscopically [4]. Additionally conductivity detection can be used, though the sensitivity of measurements for cationic surfactants is rather poor in comparison to other methods[9]. Furthermore, conductivity is obviously ill suited to detection of zwitterionic species.

This chapter describes a separation scheme which should be applicable to most surfactant species. Further, this separation is combined with a detector capable of detecting all cationic and zwitterionic surfactants that contain nitrogen. The heart of this system is the detector, a chemiluminescent nitrogen specific detector (CLND). As described in Chapter 1 the CLND allows for the specific detection of almost any nitrogen containing species, with a universal response factor dependant upon the amount of nitrogen in each analyte.

HPLC coupled to CLND has been used previously for the analysis of cationic surfactants in household products[10]. However, the analysis was limited to four cationic surfactants (the separation of lauryl and myrsityl monoethanolamide and the separation of lauryl and myristyl N-methyl glucosamide). Limits of detection were obtained as low as 25 pmol nitrogen, with a linear range from 25 to 3200 pmol nitrogen. No discussion of other cationic or any zwitterionic surfactants was presented. Similarly, HPLC-CLND has also been demonstrated for ethoxyquin antioxidants[11] compounds in which the nitrogens are found in chemically similar environments to that of many cationic surfactants.

The goal of this work was to develop an HPLC methodology that would be easily adaptable to a variety of cationic and zwitterionic surfactant mixtures. Implicit within this method development is the modification of standard HPLC procedures to make them compatible with CLND. This primarily entails the elimination of any nitrogen-containing compounds from the eluent, but also includes other more subtle considerations.

3.2 Experimental

3.2.1 Chemicals and Materials

All solutions and eluents were prepared in Nanopure ultra-pure water (Barnstead, Duburque, IO, USA). Methanol used for eluents (HPLC grade) was obtained from Fisher Scientific (Nepean, ON, Canada). Barium chloride (99%) was from Aldrich (St. Louis, MO, USA) and triethylamine was from Fisher Scientific (Nepean, ON, Canada). The structures of the surfactants examined in this work are shown in Figure 3.1. Dodecyltrimethyl-ammonium bromide (DTA^+, n=11) (99%), tetradecyltrimethylammonium bromide (TTA^+, n=13) (99%) and hexadecyltrimethylammonium bromide (HTA^+, n=15) (99%) were all from Sigma (St. Louis, MO). The zwitterionic surfactants Zwittergent® 3-1 (n=0), Zwittergent® 3-8 (n=7), Zwittergent® 3-10 (n=9), Zwittergent® 3-12 (n=11), Zwittergent® 3-14 (n=13), Zwittergent® 3-16 (n=15) (all reagent grade) were from Calbiochem (La Jolla, CA, USA). The industrial surfactant mixture Rewoteric AM CAS U (coco(amidopropyl)hydroxyldimethyl-sulfobetaine) was used as received from Witco Corporation (Dublin, OH, USA). Sodium nitrite was from BDH (Toronto, ON, Canada). Argon (prepurified) and oxygen (ultra high purity) for the CLND were purchased from Praxair (Mississauga, ON, Canada).

3.2.2 Sample Preparation

All stock solutions were made to their desired concentrations in Nanopure water. Samples were prepared fresh daily in 1.5 ml centrifuge tubes and were diluted to the desired concentration with 20% methanol in water. Stock standards were kept in 15 ml Fisherbrand® sterile disposable centrifuge tubes. The centrifuge tubes are

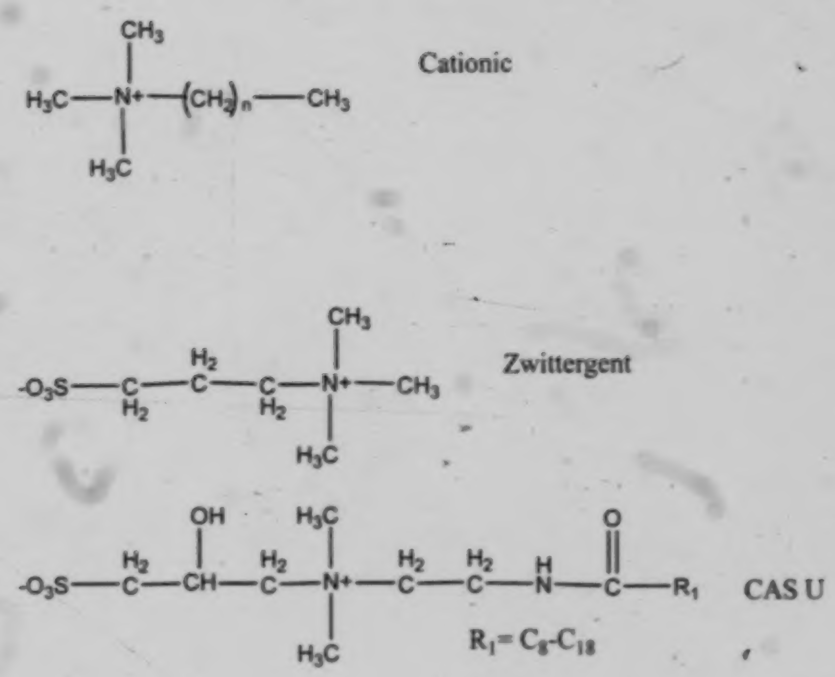

Cationic
Zwittergent
CAS U
R1= C8-C18

Figure 3.1 Structure of surfactants studied with CLND.

made of polypropylene, thus avoiding the problems of surfactant adhesion encountered by Gerhards *et al.* [12] with the use of glass containers.

3.2.3 Instrumentation

Chromatographic separations with CLND were carried out on a Waters 625 LC system (Waters Associates, Milford, MA, USA). Samples were injected using a Rheodyne 9125 injector (Rheodyne, Berkeley, CA) fit with a 20 µl polyetheretherketone (PEEK) injection loop. Connecting tubing between the injector and column was 0.005" i.d. PEEK.

All separations were performed on a 2 x 100 mm Waters Spherisorb S3 CN column. A 2.1 x 30 mm Waters Spherisorb S5 CN guard column was incorporated in some experiments. The column temperature was controlled to within 0.1 °C using an Eppendorf CH-30 (Alltech, Deerfield, IL, USA) column heater equipped with a mobile phase pre-heater and controlled by an Eppendorf TC-50. Effluent from the column was directed to the nebulizer of the CLND through an ~10 cm length of underivatized fused silica capillary (77 µm i.d., 153 µm o.d., Polymicro Technologies Incorporated, Pheonix, AZ, USA). The detector uses a zero dead volume connector (Valco Instrument Corporation Inc., Houston TX, USA) to join the capillary to the PEEK tubing from the column. In flow injection analysis studies, the column was removed such that the injector was connected directly to the detector.

The chemiluminescence detector was an Antek 8060 nitrogen detector (Antek Instruments Inc., Houston, TX, USA). The furnace temperature was 1000 °C; the argon flow was 140 ml/min with an oxygen flow of 180 ml/min and the make up flow at 140 ml/min. The reaction chamber pressure was maintained at 25 torr by the vacuum pump and the ozone flow was 30 ml/min. The analyses were done with a quartz tube containing a ceramic insert (Antek) to handle the higher salt content of the eluent. Data was acquired at 10 Hz using a National Instrument PC-6023E data acquisition board controlled using Measure software (version 2.0) (National Instruments, Austin, TX, USA) on a 486 microcomputer.

Surface tension measurements were taken using a Fisher Surface Tensiometer Model 20 (Fisher Scientific, Pittsburgh, PA, USA). The platinum-iridium ring used was cleaned in 2-butanone and then heated in a gas flame to ensure it was free of any oil residue. The glass sample beaker was also washed with 2-butanone and rinsed with water prior to measurements.

3.2.4 Barium Chloride Cleaning

The $BaCl_2$ was found to contain nitrogen impurities, presumably in the form of $Ba(NO_2)_2$ and/or $Ba(NO_3)_2$. This was determined by FIA of $BaCl_2$, with water as a mobile phase, into the CLND system. It can be seen in Figure 3.2 that the $BaCl_2$ that was not passed through the anion exchange column produced a significant nitrogen response. The nitrogen containing anions were removed through the use of an anion exchange column (4.5 x 25 cm), packed with AG® 1-X4 chloride form resin (Bio-Rad Laboratories, Richmond, CA, USA). Analysis of the $BaCl_2$ after treatment with the anion exchange column reveals that there is a significant decrease in the amount of nitrogen impurities. However as it can be seen in Figure 3.2 the "clean" $BaCl_2$ still contains some nitrogen compound(s). This impurity is potentially ammonium cation, as the anion exchange column would have exchanged any anionic nitrogen containing species for chloride. In addition the source of the chloride on the column was

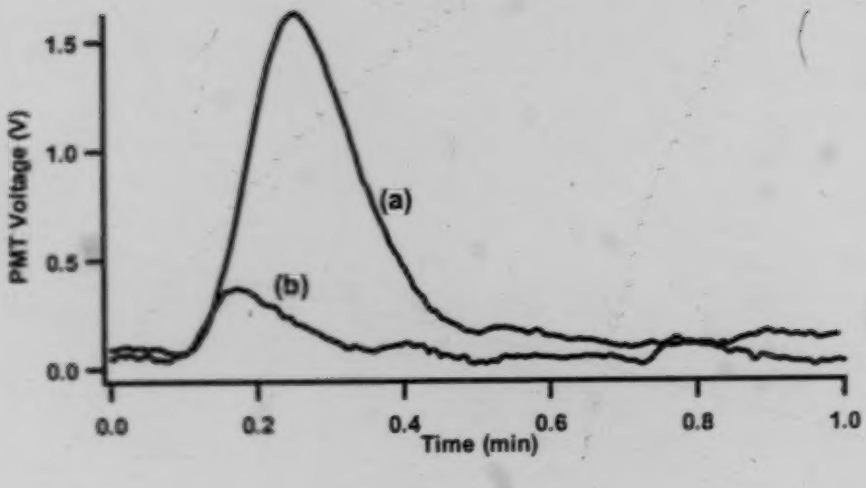


Figure 3.2: FIA analysis of $BaCl_2$ for nitrogen content with CLND, (a) before anion exchange, (b) after anion exchange; direct connection of HPLC injection module to CLND, 20 μl injection loop, 40 μM samples, eluent 100% water

hydrochloric acid, which should contain no anionic nitrogen impurities. Total nitrogen containing impurities quoted is 3 ppm ammonia. Regardless, after passage through the anion exchange column the $BaCl_2$ was sufficiently clean for our purposes.

3.3 Results and Discussion

3.3.1 Column Selection

The strong hydrophobicity of surfactants results in extremely strong retention on standard reverse-phase columns, such as those derivatized with a C-18 stationary phase. In this work, the effect of a highly retentive stationary phase is even more dramatic as strong organic eluents such as acetonitrile or THF cannot be used. Acetonitrile is incompatible with the detector due to its nitrogen content which would saturate the detector. THF, though compatible with the detector, poses a problem for the plumbing of the HPLC as the entire plumbing consists of PEEK. PEEK can swell when exposed to THF, resulting in the collapse of tubing; hence THF cannot be used with this system. The selection of cyano columns for the separation was based upon two comprehensive reviews of the analysis of surfactants [2-4] wherein the majority of the methods employed used either proprietary surfactant separation columns or cyano columns for the analysis of surfactants.

It is not immediately evident however that a cyano column would be appropriate for the separation of a mixture of surfactants. Generally, cyano columns are used as a stationary phase for normal phase separations [13]. However, cyano columns have also been successfully used in reverse-phase separations [14-17]. The retention times of hydrophobic compounds on cyano columns are similar to those on short chained reversed-phase columns (e.g. C_4 and C_8) under the same elution conditions. For instance, McCalley [18] characterized the retention of benzene on several reversed-phase columns. For a 55:45 methanol-water mobile phase, the retention factor (k) value for benzene was 1.67 on the cyano column, compared to 2.14 on a C_4 column and 3.03 on a C_8 column. The reason for this ability to retain non-polar compounds comes from the chemistry used to bind the cyano group to the silanol. In the case of cyano columns this binding is accomplished through a propyl chain [19]. When one then looks at the carbon load percentage of a cyano column we find that it is roughly half that of a C_8 column [19, 20]. This clearly illustrates that the cyano column is capable of performing reversed-phase separations of hydrophobic compounds, but that the retention factors are reduced. This is exactly what is required for the separation of surfactants, since their extreme hydrophobicity leads to near irreversible retention with standard reversed-phase columns.

3.3.2 Nitrogen Free Reversed Phase HPLC

One of the key difficulties to overcome with the use of the CLND detector is the elimination of all nitrogen from the eluent. Obviously acetonitrile cannot be used as an organic eluent. This is easily overcome through the use of other solvents. In this work, as in most other instances of reversed phase separation with CLND, methanol is an adequate substitute[10, 21, 22].

The second potential source of nitrogen in the eluent are the additives required in many mobile phases. Eluent additives are often required to tailor a separation to ensure optimum separation efficiency. Most HPLC columns are comprised of bonded phases on a silica backbone. Silica is used due to its excellent versatility, mechanical strength, efficiency and easily controlled particle size and porosity. However, the presence of silanols on the particle surface can result in broad tailed peaks in the analysis of basic samples. While considerable effort by manufacturers has been devoted to eliminating such silanol interactions, they are still very much present and of concern [18, 23, 24]. One approach to reducing the influence of the silanols is to add amines such as triethylamine, hexylamine and octylamine at milimolar concentrations to the eluent [25-28]. These amine additives will compete with the basic analytes for interactions with the free silanols. The millimolar concentration of these additives ensures that the analyte interaction with the silanols will be minimal. However the addition of amines to the eluent is incompatible with CLND as they would cause a high background signal.

Alternately, such silanol interactions could be avoided by using a polymeric stationary phase[29], but this would entail significant loss of efficiency and mechanical strength. Furthermore, the column is not the only source of silanols in the HPLC-CLND system. The effluent from the column must pass through a nebulizer before entering the pyrolysis chamber. The tube of the nebulizer included with the CLND is a derivatized silica capillary (~10 cm) [30]. The coating is unknown, but is suspected to be a C_{18} phase based on communication with the sales agent. As a precaution, the capillary was replaced with a bare silica capillary of equal length. Though this avoids the excessive retention due to the long alkyl chain stationary phase, there is a drastic increase in the amount of silanols present in the flow path.

As the cationic and zwitterionic surfactants all contain quaternary amines, these charged sites will adsorb to any free silanols present. Therefore, an alternate non-nitrogen containing eluent additive was needed to prevent this adsorption.

Recently, Reta and Carr investigated the use of divalent metal cations as alternatives to amines as additives for preventing silanol interactions [25]. They found that barium chloride was comparable to triethylamine in its ability to prevent ion pairing between silanols and benzylamine. Further, barium chloride is soluble in methanol and very soluble in water [31]. Thus it may be used with methanol-water gradient based separations without fear of precipitation of the salt. Therefore, barium chloride was incorporated into the eluent for our separations of cationic and zwitterionic surfactants. The benefit of this incorporation can be seen in the separation of a mixture of DTA^+, TTA^+ and HTA^+. In the absence of Ba^{2+} in the eluent, Figure 3.3(a), no peaks were observed upon injection of a mixture of cationic surfactants. Addition of 1 mM Ba^{2+} yielded detectable peaks. However these were broad and irreproducible. Both 5 mM and 10 mM Ba^{2+} yielded good chromatographic behaviour with the three cationic surfactants eluted within 10 minutes, shown in Figure 3.3(b). With 5 mM barium a few initial injections were necessary before chromatographic behaviour such as shown in Figure 3.3(b) was observed. Indicating that though 5mM Ba^{2+} is capable of blocking silanols, there still seems to be a need to 'prime' the nebulizer before runs will be highly reproducible. Some peak tailing is still evident with the 10 mM barium used in Figure 3.3(b). This is consistent with Reta and Carr's observation that 10 mM barium gave comparable retention factors to 10 mM triethylamine, but with more residual peak tailing [25]. Further increases in the barium concentration were not practical. The presence of any non-pyrolytic compounds in the eluent necessitates frequent cleaning of the detector to ensure proper performance, as was discussed in Section 2.3.4. Their presence will also

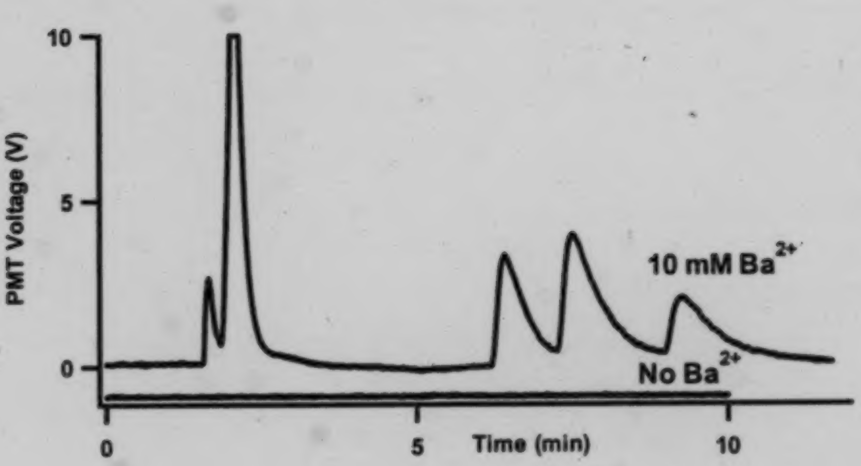


Figure 3.3: Isocratic separation of cationic surfactants DTA$^+$, TTA$^+$ and HTA$^+$. **Experimental conditions:** column, Waters Spherisorb 2.1 x 30 mm S5 CN guard column and 2 x 100 mm S3 CN analytical column; eluent, (a) 50% methanol in water (v/v) (b) containing 10 mM $BaCl_2$; column temperature 40.0 ° C in both separations.

shorten the life of the pyrolysis tube as well the membrane dryer which were problems that we encountered in this project as well as in the previous work described in Chapter 2[32]. Using 10 mM barium necessitated cleaning, approximately every two weeks depending on usage, of the tube leading to the membrane drier, of the restrictor valve and of the reaction chamber, but did not otherwise compromise the CLND performance. Over longer periods of use with such salt concentrations the pyrolysis tube would need to be cleaned or replaced to remove salt deposits to ensure proper performance, though this would likely be a monthly occurrence. It would, however, be favourable for the detector to use a more volatile barium salt (i.e., barium hydroxide, barium sulphate or barium acetate) these salts are too insoluble in methanol-water mixtures to be suitable in the eluents used[31].

The lifetime of the barium coating was also investigated, for should the barium remain bound to the surface indefinitely, less barium could be used in the eluent, reducing the impact of the salt on the CLND. An eluent comprising 10 mM $BaCl_2$ and 35% methanol was used to simulate the expected eluent conditions. This eluent was passed through the injection port and nebulizer into the CLND. No column was used for the tests, for simplicity and ease of changing from one eluent to the next. The eluent was run through the system for 15 minutes at 0.2 ml/min before injections of benzylamine were performed to compare peak height/reproducibility. The injections were performed with 10 mM $BaCl_2$ in the eluent, resulting in an average peak height of 7.7 and a standard deviation of 0.2, the 5 injections were done at approximately 2 minute intervals. After these injections the eluent was changed, such that there was no $BaCl_2$, yet the percent methanol was kept the same. After 15 minutes of flowing the new eluent through the system 12 injections of the same benzylamine solution were performed. The average peak height for the injections with no Ba^{2+} in the eluent was of 6.0 with a standard deviation of 1.7. Thus a significant increase in adsorption of amines to the silanols occurs if Ba^{2+} is not present in the eluent. That is, the effect of Ba^{2+} is short lived. Therefore $BaCl_2$ was present in the eluent in all further experiments to ensure that the cationic surfactants are not retained by silanol interactions.

One unexpected discovery was that $BaCl_2$, although 99% pure, caused a significant increase in the background signal. This suggests that the $BaCl_2$ contained significant amounts of nitrogen, presumably NO_2^- and/or NO_3^- salts. Purification of the $BaCl_2$ with anion exchange as outlined in the Section 3.2.4 reduced the background signal six fold, over the background due to un-purified $BaCl_2$.

3.3.3 Cationic Surfactants

The mixture of cationic surfactants which was examined was comprised of DTA^+, TTA^+ and HTA^+, having the carbon chain lengths of 12, 14 and 16, respectively. Figure 3.4 shows a non-optimized gradient separation of these surfactants, as well as four other aliphatic amines, in a run time of 20 minutes. The resolution of the lesser retained amines could be improved by starting at a lower percentage of methanol, or by using a more gradual increase in percent methanol. Regardless, this gradient separation was performed to demonstrate the ability of the method to identify both highly and moderately hydrophobic nitrogen containing species in the same run. Unfortunately the gradient separation technique suffers from a low throughput. The separation itself is 20 minutes, however, column re-

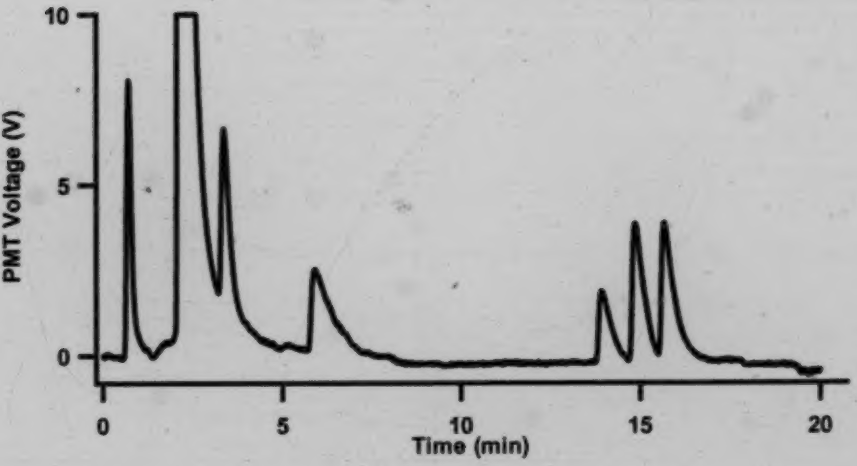


Figure 3.4: Gradient separation of triethylamine, benzylamine, heptylamine, dibenzylamine, DTA$^+$, TTA$^+$ and HTA$^+$ (in order of elution). **Experimental conditions:** Waters Spherisorb 2.1 x 30 mm S5 CN guard column and 2 x 100 mm S3 CN analytical column constant 10mM $BaCl_2$; gradient of 25% to 65% MeOH (v/v) over 10 minutes; flow rate 0.2 ml/min; 20 µl injection loop; ambient column temperature; analyte concentration varies from 100 to 400 µM.

equilibration takes at least the same amount of time. Additionally the retention times of the peaks were seen to vary based on the amount of re-equilibration which had occurred. The migration of peaks is likely due to an incomplete regeneration of the column, due to insufficient re-equilibration time[33]. Despite this, gradient separations with the CLND detector overcome the classic problem of baseline drift seen with tradition detectors as the gradient progresses[33]. Particularly with absorbance detectors the change in composition of the eluent can lead to a change in the absorbance background measurement. As a result sensitivity is decreased. However with CLND so long as there is no nitrogen in either eluent there will be no change in the background, as can be seen in Figure 3.4.

For this work however it was concluded that an isocratic method would be best for throughput and consistency when it came to the analysis of similar chain length surfactants. As can be seen in Figure 3.3, the isocratic separation of three cationic surfactants was accomplished within ten minutes. This separation was conducted with an eluent of 50% methanol by volume in water with 10 mM $BaCl_2$. The column temperature was 40.0 ° C. This isocratic separation provides at least a four fold increase in the throughput for the analysis of surfactants relative to the gradient separation. There is no interference from less hydrophobic species in these isocratic separations, since they elute well before the surfactants of interest, as can be seen with the peak due to the zwitterion Z-1.

The temperature of the isocratic separation was elevated to well above room temperature specifically in order to improve the separation of the surfactants. It is known that an increase in temperature will both increase the speed of the separation and improve the efficiency, as well as having the potential to alter selectivity[34-36]. In addition, an increase in temperature has been shown to reduce the silanol interactions with basic compounds[35, 36]. The advantages presented in elevated temperature are clearly useful for this separation, particularly in view of the fact that $BaCl_2$ is not a perfect silanol suppressor. Though the separation time could have been reduced through an increase in the amount of methanol in the eluent, this would pose two potential problems. First is the potential that, though soluble in methanol, the $BaCl_2$ could exceed its solubility should the methanol percentage become too great. Secondly, eluents of higher organic composition have the potential to explode when nebulized into the pyrolysis chamber. This can be overcome through fine adjustments to the oxygen/argon mix of the nebulizer. Nonetheless, the potential for damage of the detector is unacceptable. Due to these reasons the choice was made to elevate the temperature of the separation. Though not attempted the solubility of $BaCl_2$ could have been increased through the use of crown ethers in the eluent[37].

3.3.4 Zwitterionic Surfactants

Similar conditions were used to separate a mixture of five zwitterionic surfactants: Zwittergent® 3-8, Zwittergent® 3-10, Zwittergent® 3-12, Zwittergent® 3-14, Zwittergent® 3-16 (Z-8, Z-10, Z-12, Z-14, Z-16), the structure of which can be seen in Figure 3.1. This homologous series differs only in the length of the aliphatic chain, each one being an increase of two carbon atoms per chain over the previous form. Figure 3.5 shows the separation of these five zwitterionic surfactants along with trimethylammoniumpropanesulfonate (Z-1) which is essentially the surfactant head group. The cyano column was used with an eluent of 40% methanol in water

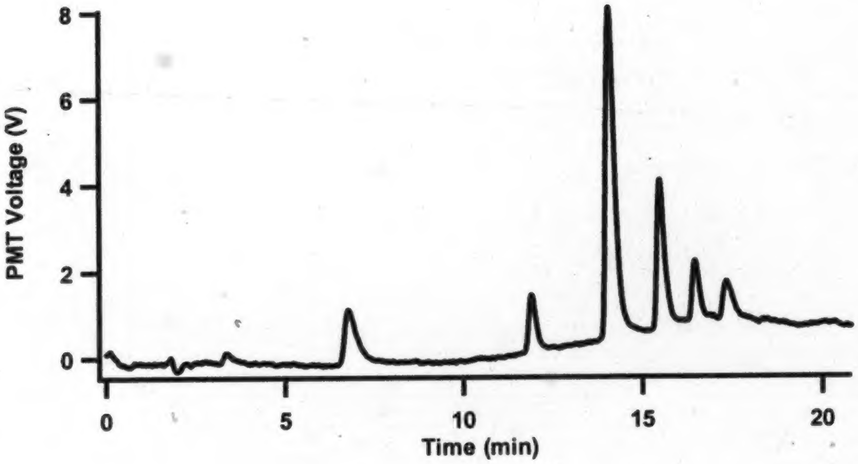


Figure 3.5: Isocratic separation of zwitterions Z-1, Z-8, Z-10, Z-12, Z-14 and Z-16. **Experimental conditions:** as in Figure 3.3 except the eluent is 40% methanol in water (v/v) with 10 mM $BaCl_2$.

with 10 mM barium chloride and a column temperature of 40.0 ° C. All components are separated with near baseline resolution within 10 minutes.

To the best of our knowledge, Figure 3.5 is the first published HPLC separation of a homologous series of zwitterionic surfactants. The detector response factor (i.e., peak area divided by molar concentration) varied only 16% over the five surfactants in Figure 3.5, listed in Table 3.1. This is slightly higher than the 6% relative standard deviation we saw with this detector for inorganic species in Chapter 2, Section 2.3.1, Table 2.2[32]. The greater variation may be the result of peak tailing leading to challenges in peak area assessment. It is evident from Figure 3.5 that an excellent choice as an internal standard for the quantification of the surfactants would be nitrate or nitrite which elutes before the Z-1 peak.

3.3.5 Linear Range and Limit of Detection

One of the advantages of CLND is its wide linear range, quoted to be 5 orders of magnitude [38, 39]. This offers a great advantage for industrial monitoring of any nitrogen-containing product, since this reduces the need for dilutions. However, if the critical micelle concentration (cmc) is exceeded, then surfactants are present both as free surfactants and in micelles. While micelle formation would not be expected to alter the CLND response, it may cause additional band broadening which could affect the linearity of calibrations performed using peak height. Therefore, using the isocratic method described in Section 3.3.4, the linearity of the response was tested using Zwittergent® 3-12. The cmc for Z-12 is 2-4 mM in 50 mM Na^+ (the counter ion is unspecified) in water [40]. Similarly, a plot of surface tension vs. surfactant concentration indicated that the cmc of Z-12 in 20% methanol was 3.6 mM. Thus the

Table 3.1 Comparison of response factors for the separation of zwitterionic surfactants, conditions as in Figure 3.5

	Response Factor
Z-8	342
Z-10	463
Z-12	336
Z-14	466
Z-16	433

peak height was monitored for Z-12 concentrations ranging from 50 μM to 5 mM, wherein the surfactant is present in the micelle form at the upper portion of the calibration range.

The plot of peak height vs. concentration (Figure 3.6) was linear (R^2 = 0.9974) over the entire range studied (50 μM to 5 mM) with an intercept equal to zero at the 95% confidence interval. That the response at the highest concentrations remained linear shows that an accurate analysis of the surfactant can be accomplished even when its concentration exceeds the cmc.

Under the conditions discussed in Sections 3.4 and shown in Figure 3.5, the limit of detection for Z-12 is 8 μM. This is comparable with the previously reported detection limit of 1 ng-N (approximately 3 μM for compounds containing a single nitrogen), observed for a variety of compounds [32]. It is not surprising that this is somewhat higher a value, as the peak shape does suffer some asymmetry due to the silanol effect. As the limit of detection for CLND is based on the amount of nitrogen in the analyte molecule, the detection limit for other zwitterionic and cationic surfactants would also be in the single-digit micromolar range. The detection limits will improve with shorter hydrophobic chain lengths, due to improved peak shapes.

3.3.6 Gradient Separation of Zwitterionic Surfactants

One of our goals in developing this method was to be able to determine the composition of an industrial surfactant mixture known as Rewortic CAS U (Figure 3.1c). This surfactant mixture has been used by our research group to control the electroosmotic flow (EOF) in capillary electrophoresis [41-43].

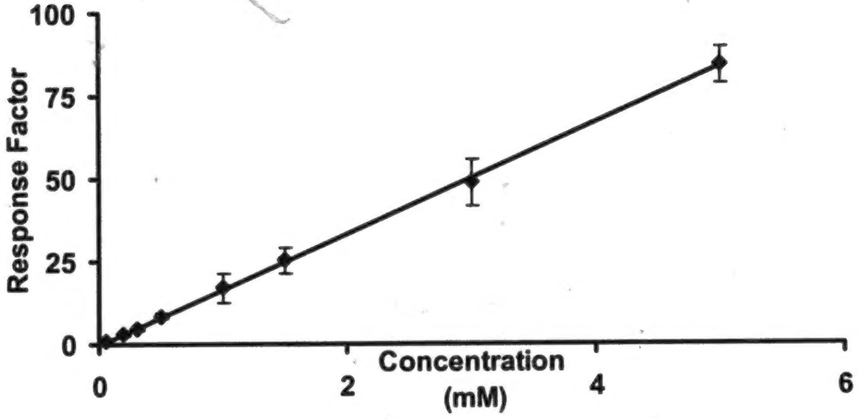


Figure 3.6: Linear regression plot for Z-12 surfactant concentrations 50 μM-5 mM. Error bars represent one standard deviation. **Experimental conditions:** Waters Spherisorb 2.1 x 30 mm S5 CN guard column and 2 x 100 mm S3 CN analytical column; all runs were isocratic, 45% methanol (v/v); 5 mM $BaCl_2$; 20 μl injection loop; 0.2 ml/min flow rate; minimum 3 replicate injections per data point.

It was known that the surfactant was synthesized using coconut oil as a starting material. The nature of the surfactant source will affect the chain lengths available as well as the distribution of the different lengths. Products from palm oil are mostly C_{16} and C_{18} with trace amounts of C_{14}. Coconut oils however produce chain lengths from C_6 to C_{18} (in two carbon increments) [44]. Therefore in knowing that the surfactant mixture can contain these possible chain lengths the analysis was facilitated.

The only previous analytical method reported for monitoring of a similar coconut derived surfactant, cocamidopropylbetaine (CAPB), used HPLC ESI-MS [28]. For their analysis the surfactants were separated, with the C_{14} form eluting after 30 minutes, by reversed-phase HPLC. The ESI analysis was performed in both the positive and negative ion modes. The MS analysis revealed the formation of dimer and trimer clusters from the ESI source in both modes, which can be problematic for analysis. Formation of doubly charged species and cleaved stable fragments were also observed. Clearly the analysis can be accomplished, but is prone to challenges from inorganic salts in the positive ion mode, which can be overcome using the negative ion mode. However, the negative ion mode is less sensitive.

Given the wide range of hydrophobicity in this surfactant mixture, gradient elution was used. The gradient was linear from 25% to 65% methanol over a ten minute period, with a constant 10 mM barium chloride. It should be noted that with this HPLC system that the dwell time of the eluent before it reaches the column, at this flow rate, is approximately 9 minutes. Thus the first 9 minutes of this separation were performed under isocratic conditions, equivalent to the initial gradient conditions. Figure 3.7 shows the resultant chromatogram. This clearly shows a good separation (resolution ≥ 1.9) of all the nitrogen containing components of the surfactant, with no evidence of overlapping peaks. Thus a clear identification of the components can be made.

In Figure 3.7, the C_{12} is the most abundant homolog followed by the C_{14}. Further, assuming equimolar response, their relative abundances are 49.9 and 18.8% respectively, which can be seen in Table 3.2. These are in good agreement with the 48.0 and 19.0% expected from a coconut oil base [44]. Again, assuming equimolar response, the average molecular weight for the mixture was determined to be 428 g/mol. This is comparable to the 450 g/mol obtained for CAS U through a gas chromatographic analysis [42]. The agreement between these two methods illustrates how well this system works for the analysis of the composition of an unknown surfactant mixture.

3.4 Conclusions

The universal response of the CLND allows for the analysis and quantification of both cationic and zwitterionic surfactants without the need for any post column reagents or significantly different chromatographic conditions. We have illustrated two highly effective separation techniques for the separation of both surfactant forms, allowing for high versatility, without the need for any modification of the detection system. Finally the limit of detection of these surfactants is in the low micromolar range, with a linear range (R^2= 0.9981) which remains linear even when the concentration of the surfactant exceeds the cmc. Thus, HPLC-CLND is a powerful methodology for determination of cationic and zwitterionic surfactants.

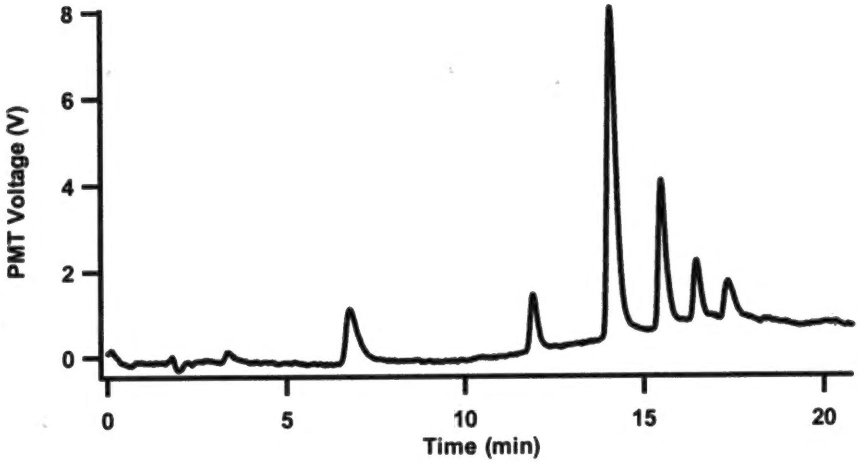


Figure 3.7: Gradient separation of the industrial zwitterionic surfactant mixture Rewoteric AM CAS U. **Experimental conditions:** Waters Spherisorb 2 x 100 mm S3 CN analytical column, CLND detection, linear gradient of 25% to 65% methanol in water (v/v) over 10 minutes, constant 10 mM $BaCl_2$; 20 µl injection loop; 0.2 ml/min flow rate; ambient column temperature; analyte concentration ~ 200 µM (above cmc).

Table 3.2 Composition of CAS U based on peak area analysis of the separation in Figure 3.7

Chain Length	**Percent Composition**	**Molecular Weight Composition**
C-8	9.5 %	35.9 g/mol
C-10	7.0 %	28.5 g/mol
C-12	51.5 %	224.4 g/mol
C-14	20.0 %	92.9 g/mol
C-16	7.1 %	35.1 g/mol
C-18	4.9 %	25.3 g/mol
Total	100 %	442.1 g/mol

3.5 References

(1) Vogt, C.; Heinig, K. *Fresen. J. Anal. Chem.* **1999**, *363*, 612-618.

(2) Morelli, J. J.; Szajer, G. *J. Surfact. Deterg.* **2000**, *4*, 75-83.

(3) Morelli, J. J.; Szajer, G. *J. Surfact. Deterg.* **2000**, *3*, 539-552.

(4) Schmitt, T. M. *Analysis of Surfactants*; Marcel Dekker Inc.: New York, 1992.

(5) Nakae, A.; Kunihiro, K.; Muto, G. *Journal of Chromatography* **1977**, *134*, 459-466.

(6) Helboe, P. *Journal of Chromatography* **1983**, *261*, 117-122.

(7) Church, W. H.; Chiang, H. T. *Journal of Capillary Electrophoresis* **1997**, *4*, 261-268.

(8) Kawase, J.; Tsuji, K.; Yasuda, Y.; Yahima, K. *J. Chrom.* **1983**, *267*, 133-148.

(9) Suortti, T.; Sirvio, H. *Journal of Chromatography* **1990**, *507*, 421-425.

(10) Truchan, J.; Rasmussen, H. T.; Omelczenco, N.; McPherson, B. P. *J. Liq. Chrom. Rel. Tech.* **1996**, *19*, 1785-1792.

(11) Brannegan, D.; Ashraf-Khorassani, M.; Taylor, L. T. *J. Chrom Sci.* **2001**, *39*, 217-221.

(12) Gerhards, R.; Schulz, R. *Tenside Surfact. Det.* **1999**, *36*, 300-307.

(13) Majors, R. E. *J. Chrom. Sci.* **1980**, *18*, 488-511.

(14) DeSmet, M.; Hoogewijs, G.; Puttemans, M.; Massart, D. L. *Anal. Chem.* **1984**, *56*, 2662-2670.

(15) Okusa, K.; Tanaka, H.; Ohira, M. *J. Chrom. A* **2000**, *869*, 143-149.

(16) Smith, R. M.; Miller, S. L. *J. Chrom.* **1989**, *464*, 297-306.

(17) Snyder, L. R.; Glajch, J. L.; Kirkland, J. J. *Practical HPLC Method Development*; Wiley: New York, 1988.

(18) McCalley, D. V. *J. Chrom. A* **1999**, *844*, 23-38.

(19) Kaczmarski, K.; Prus, W.; Kowalska, T. *J. Chrom. A* **2000**, *869*, 57-64.

(20) Waters Corporation *Waters chromatography columns and supplies catalog 1999-2000*, 1999.

(21) Fujinari, E. M.; Courthaudon, L. O. *J. Chrom.* **1992**, *592*, 209-214.

(22) Fujinari, E. M.; Manes, J. D. *J. Chrom. A* **1997**, *763*, 323-329.

(23) Rogers, S.; Dorsey, J. *J. Chrom. A* **2000**, *892*, 57-65.

(24) Stella, C.; Rudaz, S.; Veuthey, J.; Tchapla, A. *Chromatographia* **2001**, *53*, S113-S131.

(25) Reta, M.; Carr, P. W. *J. Chrom. A* **1999**, *855*, 121-127.

(26) Petrovic, M.; Barcelo, D. *Anal. Chem.* **2000**, *72*, 4560-4567.

(27) Parris, N. *J. Liq. Chromatogr.* **1980**, *3*, 1743-1751.

(28) Eichhorn, P.; Knepper, T. P. *J. Mass Spect.* **2001**, *36*, 677-684.

(29) Alltech Associates Inc. *Alltech Chromatography Sourcebook*, 2000.

(30) Borny, J. F.; Personal Communication, 2001.

(31) Merk Corporation, Ed. *The Merk Index an encyclopedia of chemicals, drugs and biologicals*; Merk & CO., Inc., 1989.

(32) Lucy, C. A.; Harrison, C. R. *J. Chrom. A* **2001**, *920*, 135-141.

(33) Dolan, J. W.; Snyder, L. R. *Troubleshooting LC Systems*
A Comprehensive Approach to Troubleshooting LC Equipment and Separations, 1st ed.; Humana Press: Clifton, New Jersey, 1989.

(34) Guillarme, D.; Heinisch, S.; Rocca, J. L. *Journal of Chromatography A* **2004**, *1052*, 39-51.

(35) Greibrokk, T.; Andersen, T. *Journal of Chromatography A* **2003**, *1000*, 743-755.

(36) Tran, J. V.; Molander, P.; Greibrokk, Y.; Lundanes, E. *Journal of Separation Science* **2001**, *24*, 930-940.

(37) Chen, Q. J.; Hou, X. L.; Yu, Y. X.; Dahlgaard, H.; Nielsen, S. P. *Analytica Chimica Acta* **2002**, *466*, 109-116.

(38) Borny, J. F. *Antek 8060 Manual*; Antek Industries, 1998.

(39) Yan, X. *J. Chrom. A* **1999**, *842*, 267-308.

(40) Calbiochem-Novabiochem Corporation; Zwittergent Test Kit literature: La Jolla California, 2000.

(41) Baryla, N. E.; Lucy, C. A. *Anal. Chem.* **2000**, *72*, 2280-2284.

(42) Yeung, K. K.-C.; Lucy, C. A. *Anal. Chem.* **1997**, *69*, 3435-3441.

(43) Yeung, K. K.-C.; Lucy, C. A. *J. Chrom. A* **1998**, *804*, 319-325.

(44) Reck, R. A. *J. Am. Oil. Chem. Soc.* **1985**, *62*, 355-365.

Chapter 4 Tetrabutylphosphonium Based Ion Pair Chromatography for the Separation of Metal Cyanide Complexes with CLND Detection

The standard methodology for the separation of metal cyanide complexes is ion pair chromatography which utilizes tetrabutylammonium cation as the ion pairing agent (IPA)[1-7]. In this Chapter the use of the chemiluminescent nitrogen detector (CLND) is explored for the detection of these nitrogen rich complexes. With the exception of azide compounds (Chapter 2) this highly specific detector provides a universal response to compounds, based on the amount of nitrogen present. However as CLND detects virtually all forms of nitrogen this precludes the use of nitrogen-containing compounds in the eluent, particularly the ion pairing agent. This chapter focuses on the application of a novel ion pairing agent, tetrabutylphosphonium (TBP), for the separation of metal cyanide complexes.

4.1. Introduction

The environmental presence of metal cyanide complexes is cause for concern due to their inherent toxicity. The toxicity of the zinc complex exceeds that of free cyanide, which is comparable to the nickel complex[8]. The source of metal cyanide complexes in the environment include industrial by-products, waste from paper de-inking and gold mining[8-11]. Due to the inherent toxicity of the complexes and their persistence significant research has been put into the development of effect biodegradation methods[9, 11]. The metal cyanide complexes are anionic species, of the general form; $M(CN)_n^{y-}$ where the metal (M) is commonly a heavy or precious metal, complexed by n=2-6 cyanide ions and with a net charge or -1 to -4. The ability to both identify and quantify these complexes is of great interest both with regard to environmental quality and mining operations.

Previous methods for the separation and determination of these complexes have been achieved primarily through liquid chromatography using ion-pair chromatography [1, 3, 6, 12] (IPC), although both capillary electrophoresis [13, 14] and ion chromatography[15] have also been employed. Typically UV detection at 214 or 215 nm is used, however, the metal cyanide complexes exhibit a range of response factors at this wavelength. Though all complexes of interest can be detected, the absorption maxima of the complexes varies from 199-215 nm[5]. A number of detection schemes that have been explored for metal cyanides including indirect, UV[15], fluorescence[6], Raman spectroscopy [6]and inductively coupled plasma[16]. Yet none of these methodologies are capable of providing an equimolar response factor for all the complexes.

My research focuses on the use of an alternative detection scheme, one that can provide a proportional response for all metal cyanide complexes. The analysis is performed through ion-pair liquid chromatography coupled to CLND. As detailed in Chapter 1, this detector allows for the universal analysis of virtually all nitrogen containing species while at the same time maintaining a selective response for only nitrogen containing species.

In Chapter 2 I showed that the combustion conditions pyrolysed cyanide and allowed for its sensitive and accurate quantification through CLND [17]. Therefore it is expected that the use of CLND for the analysis of metal cyanide complexes should prove to be equally successful. The analysis of metal cyanide complexes through

CLND offers a number of distinct advantages over current analysis methods. Quantification is facilitated by the universal response factor of the detector. Since the signal is proportional to the concentration/amount of nitrogens in the analyte, the quantification can easily be achieved through the use of a single internal standard, such as ammonium cation. Additionally with the complexes having 2-6 cyanide ligands, the sensitivity of this detection scheme is expected to surpass that which has been achieved through more traditional analysis techniques.

The separation of metal cyanide complexes however poses a unique problem when the desired detector is a CLND. Due to the range of analyte charges, from –1 to –4, ion chromatography is ineffective at producing a successful separation of all the metal cyanide complexes. Therefore the separation technique of preference has been ion-pair chromatography [1, 2, 5-7]. In all previous methods the separation was achieved using a tetrabutylammonium (TBA) salt as the ion-pair agent. The mechanism of IPC, as described in Chapter 1, results in a dynamic equilibrium of the IPA with respect to the stationary phase and mobile phase. This causes the reversed phase column to act as a low capacity, highly tuneable, ion-exchange column, allowing for the elution of highly charged ionic species while maintaining good peak efficiency.

With CLND the need to eliminate nitrogen from the eluent is paramount. Any eluent source of nitrogen will generate a large background signal; consequently increasing the limits of detection that can be achieved by the detector. Therefore it is not possible to use tetrabutylammonium cation as the IPA, and an alternate hydrophobic cation must be employed. In this Chapter I use TBP salts as the IPA. TBP is ideally invisible to the chemiluminescent detector. Another advantage of using TBP is that it is commercially available. In Chapter 5, another non-nitrogen containing IPA, tributylsulphonium cation, which is not commercially available, will be explored.

4.2 Experimental

4.2.1 Chemicals

Aqueous solutions were prepared with Nanopure ultra-pure water (Barnstead, Duburque, IO, USA). HPLC grade methanol was from Fisher (Nepean, ON, Canada). Tetrabutylphosphonium bromide (98%) was from Aldrich (Milwaukee, WI, USA), orthophosphoric acid (85%) was from Caledon (Georgetown, ON, Canada), sodium hydroxide 10 N solution (30% w/w) was from Fisher. The analytes potassium ferricyanide (99%) and potassium ferrocyanide (99%) were from Sigma (St. Louis, MO, USA), the analytes potassium dicyanoaurate (I) (98%), potassium dicyanoargentate(I) and potassium hexacyanocobaltate (III) all came from Aldrich. Cupric sulphate (99%) and sodium cyanide (ACS) were from Sigma (St. Louis, MO, USA) and nickel sulphate (ACS) was from Fisher. Gases required for the CLND were argon (prepurified) and oxygen (ultra-high purity), which were from Praxair (Mississauga, ON, Canada).

4.2.2 Sample Preparation

All stock samples of the metal cyanide complexes were stored in Nalgene containers and kept in the dark. All samples were prepared using Nanopure ultra-pure water. Analysis samples were prepared fresh by dilution of the stock solution into 0.75 ml polypropylene snap ring vials from Rose Scientific Ltd. (Edmonton, AB, Canada).

Stock solutions of Cu (I) and Ni (II) cyanide complexes were made following the procedure of Haddad *et al.* [14]. This preparation called for the mixing of either copper (II) or nickel (II) sulphate of the desired stock concentration with a fourfold excess of sodium cyanide. When working with cyanide caution must be taken at all times due to its inherent toxicity. Avoid acidification of cyanide solutions and perform all reactions in a well ventilated environment. Use appropriate care for the disposal of all cyanide waste.

4.2.3 Cleaning of Tetrabutylphosphonium Salts

The TBP was found to contain nitrogen impurities, presumed to be nitrate and nitrite salts, as was seen with barium chloride, Chapter 3.2.4. The presence of nitrogen impurities was confirmed through FIA of the TBP bromide salt, with water as a mobile phase, into the CLND system. Figure 4.1 illustrates the signal intensity seen through FIA for a 100 μM sample of TBPBr. The nitrogen containing anions were removed through the use of an anion exchange column (4.5 x 25 cm), packed with AG® 1-X4 chloride form resin Bio-Rad Laboratories (Richmond, CA, USA). Analysis of the resultant TBP chloride, the product of the anion exchange column reveals that there is a significant decrease in the amount of nitrogen impurities, Figure 4.1. However as it can be seen in Figure 4.1 the "clean" TBPCl still contains some nitrogen compounds. Further discussion pertaining to the source of the signal seen in the "clean" TBPCl can be found in Section 4.3.1.

4.2.4 Instrumentation

For this study all separations were performed using an Altima C18 column from Alltech (Deerfield, IL, USA). The column was 150 x 2.1 mm with C18 bonded

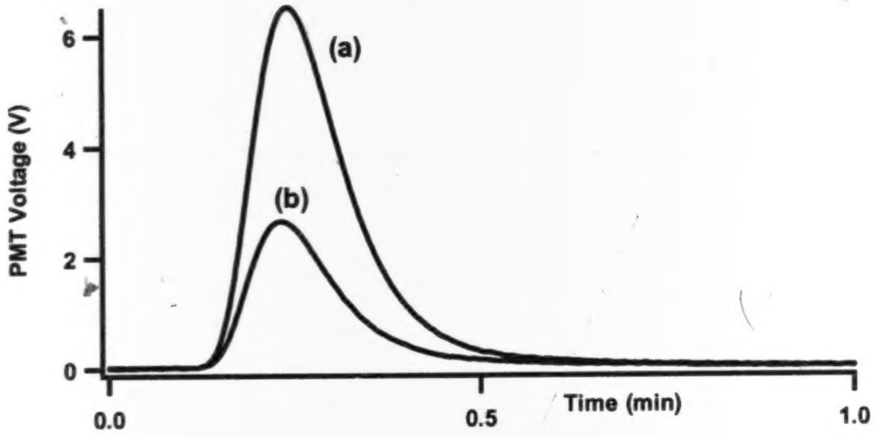


Figure 4.1 Comparison of CLND response from 100 µM TBP before (a) and after (b) cleaning with anion exchange column. **FIA Conditions:** 50% methanol, 0.2 ml/min, 20 µl injection

phase silica particles of 5 μm diameter. The column temperature was regulated using an Eppendorf CH-30 (Alltech) column heater equipped with a mobile phase pre-heater and controlled by an Eppendorf TC-50. The column heater provided control of the column temperature to within 0.1 °C. Chromatographic separations with CLND were carried out on a Waters 625 LC system Waters Associates (Milford, MA, USA). Samples were injected using a Rheodyne 9125 injector Rheodyne, (Berkeley, CA, USA) fit with a 20 μl polyetheretherketone (PEEK) injection loop. Connecting tubing between the injector and column was 0.005" i.d. PEEK.

Effluent from the column was directed to the nebulizer of the CLND through a ~10 cm length of underivatized fused silica capillary (77 μm i.d., 153 μm o.d., Polymicro Technologies Incorporated, Pheonix, AZ, USA). The detector uses a zero dead volume connector (Valco Instrument Corporation Inc., Houston TX, USA) to join the capillary to the PEEK tubing from the column. In flow injection analysis studies, the column was removed such that the injector was connected directly to the detector.

The chemiluminescence detector was an Antek 8060 nitrogen detector (Antek Instruments Inc., Houston, TX, USA). The furnace temperature was 1100 °C; the argon flow was 140 ml/min with an oxygen flow of 180 ml/min and a make up flow comprised of argon gas at 140 ml/min. The reaction chamber pressure was maintained at 25 torr by the vacuum pump and the ozone flow was 30 ml/min. The analyses were done with a quartz tube containing a ceramic insert (Antek) to handle the higher salt content of the eluent. Data was acquired at 10 Hz using a National

Instrument PC-6023E data acquisition board controlled using Measure software (version 2.0) (National Instruments, Austin, TX, USA) on a 486 microcomputer.

4.3 Results & Discussion

4.3.1 Phosphonium/CLND Compatibility

The idea of using tetrabutylphosphonium (TBP) with CLND was based entirely the assumption that TBP would yield no chemiluminescence background. However as I had learned with barium chloride in Chapter 3, salts are often contaminated with trace amounts of nitrogen that are detectable by the CLND. Thus, the purity of the commercial TBP was checked using FIA analysis, whereby it was evident that the TBP contained nitrogen. Figure 4.1 shows a signal intensity that is equivalent to 0.2 mM of nitrogen in a 100 mM solution of TPB supplied by Aldrich. Cleaning of the TBP salt using the same procedure as outlined in Section 3.2.4 decreased the FIA peak area of the TBP, from 620 to 260 for a 100 mM solution. The new signal intensity is equivalent to 0.08 mM nitrogen in a 100 mM TBP solution, less than 0.1% nitrogen.

The residual signal after cleaning could be due to two possible sources. There could be cationic nitrogen species present which would not be removed by the anion exchange resin. Alternately phosphonium can produce luminescence through a mechanism similar to the nitrogen luminescence detailed in Section 1.2. The phosphorus luminescent reaction has been used for the determination of both phosphorus[18] and phosphate[19]. In both instances the phosphorus is combusted to form phosphine, depicted in Equations 4.1 and 4.2, which is subsequently reacted with ozone resulting in a luminescent reaction, under similar conditions to those with the CLND. No products were predicted for the reaction of phosphine and ozone (Eq. 4.2)

$$4H_3PO_4 + \text{heat} \rightarrow PH_3 + 3H_3PO_4 \tag{4.1}$$

$$PH_3 + O_3 \rightarrow ?? + h\nu \tag{4.2}$$

The emission from this reaction is broad and falls within the 600-900 nm range utilized for nitrogen analysis in CLND. It was however seen that the maximum intensity wavelength of the signal from the phosphine-ozone reaction was concentration dependant[19]. At 20 ppm the emission maxima shifts down to 530 nm, with emission spectra extending out only to 700 nm. Therefore it is favourable to use the lowest concentrations of TBP possible with CLND to minimize any potential background signal. This also helps minimize any background signal due to nitrogen impurities in the phosphonium salt.

At this time the source of the signal from the purified TBP is undetermined. The presence of cationic nitrogen could be confirmed through a separation of the salt, either through ion exchange or ion pair chromatography[20]. Regardless, at 25 mM TBP the background signal is only 0.67 V ($\leq$ 4.5% RSD). The zeroed background signal has a standard deviation of only 0.03 V. As such this background did not interfere significantly with the determination of the metal cyanide complexes.

4.3.2 Separation of Metal Cyanides with TBP

In numerous studies of the separation of metal cyanides with TBA, the elution order most frequently followed is from highest to lowest charge[1, 5, 6]. This elution order was observed over wide range of TBA concentration (7.25-60 mM) and buffer salt concentrations (0-150 mM). With TBP a significantly different elution

Table 4.1 Comparison of Elution Orders of Metal Cyanide Complexes With TBP and TBA

Elution Order[a]	TPB[b]	TBA[c]	TBA[d]
1	$Ag(CN)_2^-$	$Mo(CN)_8^{4-}$	$Cu(CN)_4^{3-}$
2	$Cu(CN)_4^{3-}$	$Cu(CN)_4^{3-}$	$Ni(CN)_4^{2-}$
3	$Co(CN)_6^{3-}$	$Co(CN)_6^{3-}$	$Au(CN)_2^-$
4	$Cr(CN)_6^{3-}$	$Fe(CN)_6^{3-}$	$Co(CN)_6^{3-}$
5	$Ni(CN)_4^{2-}$	$Ni(CN)_4^{2-}$	$Fe(CN)_6^{3-}$
6	$Au(CN)_2^-$	$Co(CN)_6^{3-}$	$Fe(CN)_6^{4-}$
7	$Fe(CN)_6^{4-}$	$Pd(CN)_4^{2-}$	
8	$Fe(CN)_6^{3-}$	$Pt(CN)_4^{2-}$	
9		$Au(CN)_2^-$	

a. 1 is the first eluted component, and 9 is the last eluted.

b. This work. 25 mM TBPCl, 50% methanol, 0.3 ml/min, Alltech C18 column (150 x 2.1 mm, 5 μm), 35.0 °C

c. From reference [1]. Condition: 150 mM H_3PO_4, 60 mM TBAOH, 25% acetonitrile, 2.34 mM $NaClO_4$, pH=7.64, 1.0 ml/min, Nova-Pak C_{18}, guard (5.0 x 3.9 mm) and analytical column (150 x 3.9 mm)

d. From reference [7]. Conditions: 4 mM TBAOH, 35% acetonitrile, 1.25 mM NaH_2PO_4, pH = 8, 1.0 ml/min, Du Pont-Zorbax R_x-C_{18} (150 x 4.6 mm, 5 μm) order was observed for the metal cyanide complexes. Table 4.1 lists the elution order of the metal cyanide complexes with both TBA and TBP as the IPA. This change in elution order with TBP was surprising, as previous studies with TBP did not reveal a change in the elution order[21]. Also, there was no discernable pattern in the elution order. The existing studies on the retention of metal cyanides were examined to discern if there was any known mechanism that could account for the difference in retention order.

The most complete study on the effect of eluent additives on the retention of metal cyanide complexes was done by Huang *et al.*[1]. They investigated the impact of both IPA concentration (5-90 mM) and perchlorate concentration (0.32-5.62 mM) at a constant phosphate concentration of 150 mM. Increasing the IPA concentration revealed retention factor maximums occurred at IPA concentrations between 30-60 mM for most complexes. The maximum retention factors observed ranged from k'~ 8 for $Cu(CN)_4^{3-}$ to k'~ 60 for $Cr(CN)_6^{3-}$. However the retention of $Cr(CN)_6^{3-}$ and $Co(CN)_6^{3-}$ increased steadily as the concentration of TBA increased, ultimately reaching k' ~ 60 for $Cr(CN)_6^{3-}$ and k' ~ 24 for $Co(CN)_6^{3-}$ at 90 mM TBA.

In addition to the changes in the TBA concentration, the influence of the perchlorate ion was also investigated, over a range of 0.32 to 5.62 mM. This was done with the hope of elucidating the ion exchange character of the retention mechanism. What was seen was that most of the complexes experienced a decrease in retention with increases in concentration of perchlorate. Exceptions were noted for the copper (I) and gold (I) complexes which showed almost no change in retention over the range of perchlorate studied.

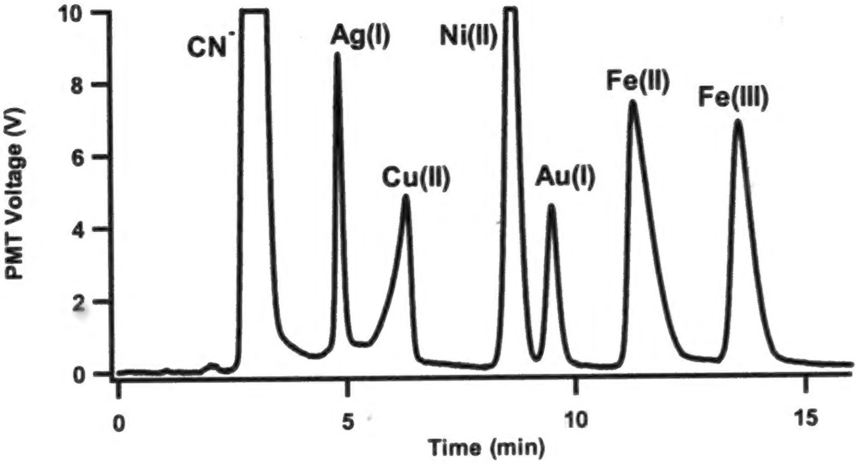


Figure 4.2 Separation of metal cyanide complexes with CLND **Elution Conditions:** 20 mM TBPCl, 50% methanol, 0.3 ml/min, Alltech C18 column (150 x 2.1 mm, 5 μm), 35.0 °C, all complexes 100 μM

Despite Huang *et al.*'s extensive study of metal cyanides separations with TBA [1], the elution orders that were obtained were unlike those I observed with TBP (Figure 4.2). Table 4.1 compares the elution order of the metal cyanide complexes with both TBA and TBP as the IPA. With the TBA work of Huang *et al.*[1] the trend is seen that the least retained compounds are those of highest charge. This is similar to the results seen by several other authors doing separations of metal cyanide complexes with TBA[4-6, 22]. The only exception found in the literature was in the work of Giroux and Barkley[7]. Though they also used TBA they saw a distinctly different elution order (rightmost column in Table 4.1) to that seen by others working with TBA. The elution order achieved by Giroux and Barkley does not correlate to the charge of the complexes. To address this difference in elution order Giroux and Barkley showed that changing the ionic strength of the eluent led to selectivity changes and some changes in elution order[7]. Giroux and Barkley concluded that the differences in the ionic strength and column chemistry were responsible for the differences in retention order. It is of note that the experimental conditions used by Giroux and Barkley were similar to those of my current experiment, wherein neither study involved increases in the ionic strength beyond that due to the IPA. The only differences between the eluents in the two studies, other than the nature of the IPA, are the concentration of IPA used and the organic modifier (methanol in my work in place of acetonitrile). The percentage of methanol used in my study, 50%, is a somewhat stronger eluent than the 35% acetonitrile used by Giroux and Barkley[23]. Though the strength of the organic modifiers is not the same, it will be shown in Chapter 5 that the amount of organic modifier is unlikely to result in a selectivity change. The concentrations of the IPA used also differed between the two studies. My study utilized 20 mM TBP, whereas Giroux and Barkley used 4 mM TBA. It was shown in the work of Huang *et al.*[1] that increasing the concentration of the IPA will primarily result in an increase in retention for all the cyanide complexes. Though Huang *et al.*[1] saw some selectivity changes, those changes do not coincide with the differences between my work and that of Giroux and Barkley. Therefore though it is possible to achieve alterations in selectivity based upon changes in the concentration of the IPA and the ionic strength, the selectivity which is achieved herein using TBP is unique.

4.3.3 Chromatographic Behaviour

Figure 4.3 shows the separation of eight metal cyanide complexes within 15 minutes using TBP. This represents a significant improvement in analysis time, which previously had been on the order of 30-40 minutes[1,5]. Other separations have been performed with close to 15 minute retention times, though this either involved fewer analytes (<6) [2,3,6,24], or suffered from poor resolution (R_s <0.8) of several peaks[7]. Under the optimal conditions (Fig. 4.3) the minimum resolution was 1.4 (between the nickel and gold complex). Also, $Cu(CN)_4^{3-}$ experiences strong fronting, which at high concentrations will extend into the $Ag(CN)_2^-$ peak. This distortion in the peak shape may be due to changes in the oxidation state of the copper[25], altering the retention characteristics.

In particular the retention times of all analytes decreased, on average 3.3%, with subsequent runs. This appears to have been due to some instability of the column, as after working with the column for a month and a half all peaks began to

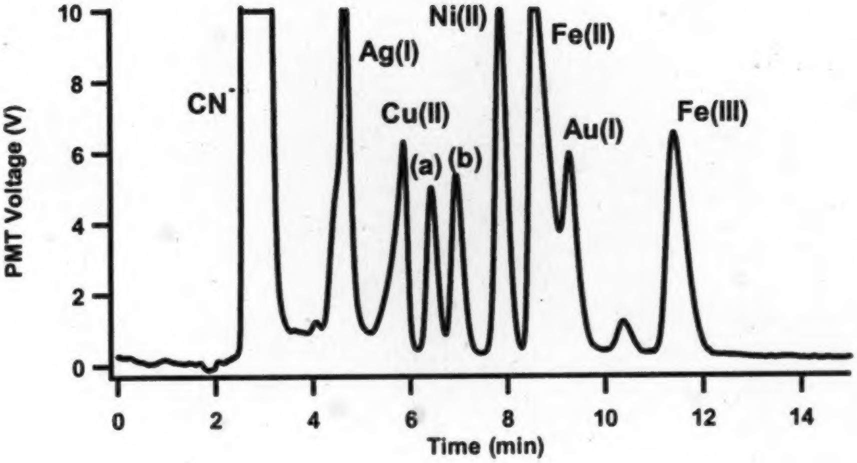


Figure 4.3 Separation of 8 metal cyanide complexes within 15 minutes, with CLND; peak (a) Co(III), peak (b) Cr(III) **Elution Conditions:** 25 mM TBPCl, 50 % methanol, 0.3 ml/min, Alltech C18 column (150 x 2.1 mm, 5 μm), 35.0°C, all complexes 100 μM

co-elute. The cause of the degradation is remains unknown. It is suspected that the use of the basic form of the IPA, tetrabutylphosphonium hydroxide, early in the study may have contributed to shorter than expected lifetime. The decreased retention that occurred as the column degraded did have some influence on the selectivity. This was particularly evident in the decreased retention of the iron complexes. In particular the decrease in the retention time of $Fe(CN)_6^{4-}$ complex was on average 6.8%. This loss of retention ultimately resulted in $Fe(CN)_6^{4-}$ co-eluting with $Au(CN)_2^-$. It is clear that not all metal cyanides are retained by these hydrophobic interactions to the same extend as $Fe(CN)_6^{4-}$. Complexes such as those of gold, nickel and silver did not experience as drastic a decrease in retention times. This also supports the conclusion of Giroux and Barkley, that the column characteristics are important to the selectivity of the separation.

4.3.4 Response Factors of Metal Cyanides

Our key interest in this work was the potential of having a detector that would provide a universal response to all of the analytes. Also, the large number of nitrogens present on each metal cyanide complex should improve upon the 3 μM detection limit achieved for pure cyanide[17]. Calibration is facilitated with a universal response factor, as quantification can be achieved through the inclusion of a known nitrogen standard in the sample. This calibration method has been used before, with nitrate or nitrite acting as standards for the analysis of ammonium in waste water[26]. The same principal can be applied to the analysis of the metal cyanide complexes, with any nitrogen compound that can be resolved on the chromatogram.

When the relative peak areas (response factors) of the metal cyanides are analyzed, Table 4.2, it is seen that the signal intensity is not universal for all complexes. This is not the first time that I have discovered a compound that has non-uniform response for the CLND[17]. In Chapter 2 I noted that the non-uniform response of azide was influenced by two factors: the chemical decomposition of azides and the concentration of azide in the pyrolysis chamber. The formation of dinitrogen gas seems rather unlikely in the instance of the thermal decomposition of the metal cyanides. The propensity for azides to form dinitrogen gas arose from the structure of the azide bonds, the multiple bonds between nitrogens facilitates the formation of dinitrogen gas. In the case of metal cyanides there is no direct bond between the nitrogens; reducing the chance of formation of the undetectable dinitrogen gas. Nor would the lack of response appear to be due to overly high concentrations of nitrogen in the pyrolysis chamber. It was shown in Chapter 2 that the concentration of azide also contributed to further attenuate the signal strength. Once again this was attributed to the formation of dinitrogen gas. Such a mechanism cannot explain the results seen with the metal cyanides. The attenuation of the azide signal occurred at a concentration 50 mM, the equivalent of 150 mM nitrogen. Yet $Ni(CN)_4^{2-}$ had the greatest signal intensity of all the metal cyanides at a concentration of 100 mM or 400 mM nitrogen. Thus it is clear that though concentration plays a factor in the formation of dinitrogen gas in the CLND, there is a need for pre-existing nitrogen-nitrogen bonds to be present prior to combustion.

Consequently the nature of the metal cyanide complexes needs to be examined to determine a possible cause for the differences in signal intensities. As stated above, the assumption was made that the CLND would provide sufficient heat to fully decompose the carbon-nitrogen triple bond, as was seen with the cyanide ion in Chapter 2. Yet as the cyanide is bound to a metal it is unclear that the carbon-nitrogen bonds are broken while the cyanide is still bound to the metal or if the metal-carbon bond must be broken before the decomposition of nitrogen can occur. The likelihood of the former possibility occurring would depend upon the impact of the metal species on the cyanide ligand. The potential exists that the bonding of the cyanide ligands to the metal could weaken the carbon-nitrogen triple bond through back bonding. However the nature of the cyanide ligand is such that it is strong π -acceptor[27]. Thus back-bonding occurs within the metal-cyanide complex with electrons donated from the metal to the ligand, maintaining the integrity of the carbon-nitrogen bond. Measurements have been made of the back-bonding in iron-cyanide complexes[28]. Measurements of the x-ray absorption spectra reveal a difference in the position of the main absorption peak. In the Fe(II) complex this peak is at a photon energy value 0.63 eV greater than is seen for the Fe(III) complex. The authors associate this difference in absorption energy with differences in back-bonding[28]. Unfortunately there is no data for the majority of the metal-cyanide complexes that can be used to gauge if the extent of back-bonding can be correlated to the signal intensities observed.

Though back-bonding data is not available for all the complexes, data exists for the dissociation factors of all the metal cyanide species of interest. When observing the bond dissociation factors there appear to be a strong correlation with the signal intensity from CLND. Table 4.2 lists the signal strength, proportional to

Table 4.2 Dissociation constant and response factor with CLND for several metal cyanide complexes.

Complex	**$pK_{dissociation}$[a]**	**Response Factor**	**One Standard Deviation**
$Ag(CN)_2^-$	20.9	440	51
$Ni(CN)_4^{2-}$	~22	536	52
$Cu(CN)_4^{3-}$	30.7	322	16
$Fe(CN)_6^{3-}$	~36	438	20
$Au(CN)_2^-$	~37	422	29
$Fe(CN)_6^{4-}$	~42	409	32

a. Reference 25, $pK_{dissociation}$ determined in water the number of nitrogens, and the dissociation constants measured for the complexes[25] for the complexes studied. A plot of the response factors vs. the bond dissociation energy is presented in Figure 4.4. There is some correlation between the overall bond dissociation energies and the signal intensity registered by the CLND. Though no literature was found on the topic it is possible that the kinetics of the reactions of the metal cyanides may provide better correlation to response factors that are seen. Unfortunately due to the variations in signal intensity due to bond energies it appears that CLND does not offer the expected advantage of an easy calibration. This is the result of the lack of a universal response factor for the metal cyanide complexes. This is exemplified by the 29% RSD for the response factors of replicate measurements of a 100 μM $Fe(CN)_6^{3-}$ complex (Table 4.3). This is almost double the RSD that was found for a combination of organic and inorganic nitrogen species in my previous work[17]. The large RSD of the response factors makes calibration difficult. Further studies are required to determine if eluent and pyrolysis conditions can be altered to provide better reproducibility.

4.3.5 Limits of Detection

The use of CLND offers great potential for the sensitive detection of metal cyanide complexes as it is a mass sensitive detection scheme. Based upon the previously achieved 3 μM detection limits for cyanide,[17] it was anticipated that the limit of detection for the metal cyanide complexes would be comparable or better. However the aforementioned irregularities in the signal intensity for the various complexes led to inherent differences in the limits of detection.

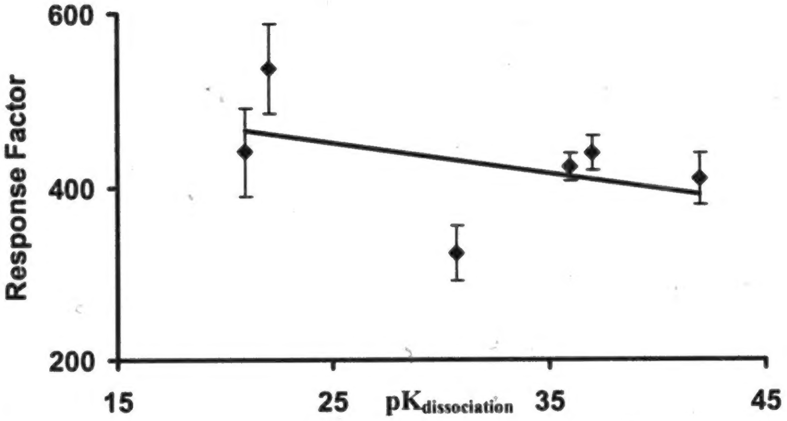


Figure 4.4 Plot of the response factor of metal cyanide complexes; Ag(I), Au(I), Ni(II), Cu(I), Fe(II) and Fe(III), versus their respective $pK_{dissociation}$ values. Error bars represent one standard deviation.

Table 4.3 CLND Response Factors for Metal Cyanide Complexes[a]

	Response/N[b]	**% RSD**
$Ag(CN)_2^-$	440	22
$Cu(CN)_4^{3-}$	322	10
$Ni(CN)_4^{2-}$	536	9
$Au(CN)_2^-$	422	14
$Fe(CN)_6^{4-}$	536	18
$Fe(CN)_6^{3-}$	438	29

a. Eluent conditions: 25 mM TBPCl, 50% methanol, 0.3 ml/min, Alltech C18 column (150 x 2.1 mm, 5 μm), 35.0 °C

b. Average response factor and %RSD (5 replicate runs) for each metal cyanide complex, corrected for the number of nitrogens per complex, with CLND detection.

Unfortunately before a complete analysis for the limit of detection was performed the CLND suffered a catastrophic failure, from which it was unable to recover. The lowest concentration of the metal cyanide complexes that were analyzed before the failure was of 100 μM. The promise of low limits of detection was evident in the response factor for $Ni(CN)_4^{2-}$, which at 100 μM went off scale with the detector gain at half the maximum. However detection limits will only be less than an order of magnitude greater than that of pure cyanide, as metal cyanidecomplexes only at best a 5 fold greater amount of nitrogen per molecule. It is anticipated, based upon the observed peak intensities, theoretical plates of the separation and noise in the baseline, that the limit of detection would be comparable to that achieved for cyanide, 3 μM. However for complexes such as $Fe(CN)_6^{3-}$, $Fe(CN)_6^{4-}$ and $Cu(CN)_4^{3-}$, which produce broad peaks, the limit of detection will be higher. The current lowest limit of detection for $Au(CN)_2^-$ was achieved by Haddad and co-workers[3], at 1.7 nM by UV detection, though the preconcentration of 3 ml of sample. However a proper comparison of the actual number of moles analyzed through each method reveals that the mole limit of detections only differ by an order of magnitude: $5x10^{-12}$ moles for Haddad and $6x10^{-11}$ moles by my method.

4.4 Conclusions

It is clear from this work that there is a limit to the universality of the CLND. Though it is capable of detecting all nitrogen species, the claim of a universal response factor has been found to have exceptions. Though the limitation of the detector for the analysis of azide compounds could be attributed to a known mechanism, the reason for the differences in response factors for the metal cyanide complexes is still unclear. It is however apparent that the metal centre of the complex has a strong influence upon the pyrolysis and requires further inquiry to determine the full nature of the influence. Initial observations show a potential correlation between the dissociation constants and the CLND response factor.

The use of phosphonium cation as an ion pairing agent compatible with CLND has been successfully demonstrated. The separation of the metal cyanide complexes with TBP illustrates its ability to be used in place of TBA. This however can necessitate further changes to the separation conditions to account for the different retentive characteristics of TBP. A study of these retention characteristics is the topic of Chapter 5.

4.5 References

(1) Huang, Q.; Paull, B.; Haddad, P. R. *Journal of Chromatography A* **1997**, *770*, 3-11.

(2) Haddad, P. R.; Rochester, N. E. *Journal of Chromatography* **1988**, *439*, 23-36.

(3) Haddad, P. R.; Rochester, N. *Analytical Chemistry* **1988**, *60*, 536-540.

(4) Haddad, P. R.; Kalambaheti, C. *Analytica Chimica Acta* **1991**, *250*, 21-36.

(5) Hilton, D. F.; Haddad, P. R. *Journal of Chromatography* **1986**, *361*, 141-150.

(6) Miralles, E.; Compano, R.; Granados, M.; Prat, M. D. *Analytica Chimica Acta* **2000**, *403*, 197-204.

(7) Giroux, L.; Barkley, D. J. *Canadian Journal of Chemistry* **1994**, *72*, 269-273.

(8) Quan, Z. X.; Bae, J. W.; Rhee, S. K.; Cho, Y. G.; Lee, S. T. *Biotechnology Letters* **2004**, *26*, 1007-1011.

(9) Ebbs, S. *Current Opinion in Biotechnology* **2004**, *15*, 231-236.

(10) Mansfeldt, T. *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde* **2001**, *164*, 637-641.

(11) Patil, Y. B.; Paknikar, K. M. *Process Biochemistry* **2000**, *35*, 1139-1151.

(12) Otu, E. O.; Byerley, J. J.; C.W., R. *International Journal of Environmental Analytical Chemitry* **1996**, *63*, 81-90.

(13) Aguilar, M.; Farran, A.; Marti, V. *Journal of Chromatography A* **1997**, *767*, 319-324.

(14) Kuban, P.; Buchberger, W.; Haddad, P. R. *Journal of Chromatography A* **1997**, *770*, 329-336.

(15) Fagan, P.; Paull, B.; Haddad, P. R.; Dunne, R.; Kamar, H. *Journal of Chromatography A* **1997**, *770*, 175-183.

(16) Marti, V.; Meinhardt, E.; Farran, A.; Cortina, J. L.; Aguilar, M. *Quimica Analytica* **2000**, *19*, 213-216.

(17) Lucy, C. A.; Harrison, C. R. *Journal of Chromatography A* **2001**, *920*, 135-141.

(18) Matsumoto, K.; Fujiwara, K.; Fuwa, K. *Analytical Chemistry* **1983**, *55*, 1665-1668.

(19) Fujiwara, K.; Kanchi, T.; Tsumura, S.; Kumamaru, T. *Analytical Chemistry* **1989**, *61*, 2699-2703.

(20) Aced, G.; Mockel, H. J.; Nelsen, S. F. *Journal of Liquid Chromatography* **1989**, *12*, 3201-3218.

(21) Wrath, L. M.; Cooper, R. S.; Fritz, J. S. *Journal of Chromatography* **1989**, *479*, 401-409.

(22) Grigorova, B.; Wright, S. A.; Josephson, M. *Journal of Chromatography* **1987**, *410*, 419-426.

(23) Snyder, L.; Kirkland, J. *Introduction to Modern Liquid Chromatography*, 2 ed.; John Wiley and Sons, Inc.: New York, 1979.

(24) Otu, E. O.; Robinson, C. W.; Byerley, J. J. *Analyst* **1993**, *118*, 1277-1280.

(25) Gerhartz, W. *Ullmann's Encyclopedia of Industrial Chemistry, 5th ed., Volume A 8*; VCH Publishers: New York, 1987.

(26) Fujinari, E. M.; Courthaudon, L. O. *Journal of Chromatography* **1992**, *592*, 209-214.

(27) DeKock, R. L.; Gary, H. B. *Chemical Structure and Bonding*; The Benjamin/Cummings Publishing Company: Meleno Park, California, 1980.

(28) Vinogradov, A. S.; Preobrajenski, A. B.; Krasnikov, S. A.; Chasse, T.; Szargan, R.; Knop-Gericke, A.; Schlogl, R.; Bressler, P. *Surface Review and Letters* **2002**, *9*, 359-364.

Chapter 5 Comparison of Selectivities of Ammonium, Phosphonium and Sulfonium Ion Pairing Agents

The use of chemiluminescent nitrogen detection (CLND) clearly requires new approaches to separation techniques. As was discussed in the earlier Chapters the need for different eluents is paramount in the use of CLND. In Chapter 4 the need for new ion pairing agents lead to the investigation of phosphonium and subsequently sulfonium. This chapter explores the fundamental behaviour of these cationic ion pair agents relative to traditional ammonium ion pairing agents. In addition, a novel approach is taken in classifying the characteristics of these ion pairing agents. Similarly, the analyte anions are classified into three distinct groups, each of which undergoes unique selectivity changes with different eluent conditions.

5.1 Introduction

Ion pair chromatography, as was described in Chapter 1, can be used for the separation of charged species. It is ideally suited for samples with analytes of widely varying charges, and is particularly amenable to analytes of higher charge. Ion pair chromatography has been used for a wide range of determinations. These include: metals[1]; arsenic speciation[2]; sulphonated compounds[3]; fatty acids[4]; food composition[5]; nucleotide-activated sugars[6]; and the analysis of pharmaceuticals, proteins and nucleic acid derivatives[7]. The versatility of ion pair chromatography allows separation conditions to be adjusted to suit the requirements of the specific determination. Factors that can be adjusted include the concentration and nature of: the counter ion, the organic modifier and the ion pairing agent (IPA). Of these variables, it has been the nature of the IPA that has been explored the least. All anion separations with ion pair chromatography have relied on ammonium-based IPAs. By not being able to select different cationic species the selectivity of the separations is limited to that provided with ammonium. This has become particularly relevant recently with the need to quantify environmental levels of perchlorate[8-11]. Though perchlorate can easily be separated from other anions retention times of perchlorate are very large, leading to a low analysis throughput. Ideally different IPAs should be available to provide alternatives for greater speed and/or selectivity.

Alternatives to ammonium IPA however are not readily available. To be suitable as an IPA a cation must possess a significant hydrophobicity, yet be soluble in aqueous media. Also the cation must be chemically stable over a range of eluent conditions. The charge on the compound should also be permanent, allowing for analysis over a range of pH values without compromising the separation capabilities. This is the case with all ammoniums ions used, which include tetraethylammonium, tetrapropylammonium and the most commonly tetrabutylammonium. The ammonium ion is however only one of several cationic functionalities that can be hydrophobic, consisting of the general structure R_nA^+. This class of compounds is referred to as the *oniums*[12]. This class includes the cations of ammonium, phosphorus, sulfur, arsenic as well as others. The substituents to the cationic center are generally alkyl chains and need not all be of the same length. Further, the degree of hydrophobicity can be tailored by adjusting the types of alkyl chains incorporated in the onium. Of the various oniums only phosphonium has been investigated previously for its properties as an ion exchanger and IPA in chromatography[13, 14]. The authors of these papers noted that polarizable anions exhibited greater retention with phosphonium IPA than with ammonium IPA. However, no further investigations were conducted. Michigami *et al.*[14] speculated that the increase in retention of polarizable anions could be due to relative electronegativities of nitrogen (3.0) and phosphorus (2.1). Upon examining the properties of sulfonium in this work it will be shown that this assumption is incorrect, as it does not explain the behaviour of sulfonium IPA based on sulfur whose electronegativity is 2.5. Phosphonium IPAs have also been used for the separation of anionic heavy-metal unithiol complexes by ion pair chromatography[15].

In this chapter the differences in the retention of common inorganic anions are examined for three different IPAs: tetrabutylammonium, tetrabutylphosphonium and tributylsulphonium. Also, this chapter characterizes these IPAs using a broad range of anions. Previous studies have been limited by the use of test anions which either were simply variants of the same type of anions (*e.g.*, acetic acids[16]) or even just a single ion (*e.g.*, adrenaline[17]). Such limited test anion sets ignore the broad spectrum of anion behaviour. Common inorganic anions have been shown to exhibit unique characteristics depending on their position in the Hofmeister series[18-21]. In this study, kosmotropic anions (iodate, chloride and nitrite), intermediate anions (bromide and nitrate) and chaotropic anions (iodide, thiocyanate and perchlorate) are used to characterize the behaviour of the tetrabutylammonium, tetrabutylphosphonium and tributylsulphonium IPAs.

To the best of my knowledge, the use and classification of such a diverse group of anions is unique in the study of ion pairing agents.

5.2 Background

5.2.1 Hofmeister Series

The Hofmeister series was initially developed to rank various neutral salts with respect to their influence on the solubility of proteins. Developed in 1888 and studied intensively since then the Hofmeister series has revealed several key characteristics of salts:

1- Their effect is most important at concentrations of 0.01 to 1.0 M.
2- Regardless of the measurement technique, the Hofmeister ranking remains the same, with occasional slight differences.
3- Not all salts effect solubility the same. Progression along the Hofmeister series leads to a reversal of effects on solubility.
4- The Hofmeister effects are predominantly influenced by the anion.
5- The effects of multiple species in solution are roughly additive.

The trend of ion behaviour with the Hofmeister series has been extensively studied and two reviews cover much of the details from the biophysical sciences research[22, 23]. The Hofmeister series has been used previously to explore more than just the influences of salts on proteins. The series has been used to explain various chromatographic retentions: the retention of cations with zwitterionic ion chromatography[24], the retention of amines on reversed phase chromatography[25], the association of inorganic anions with zwitterions in capillary electrophoresis[26] and to elucidate the mechanism of electrostatic retention in ion chromatography

[27]. Surprisingly the Hofmeister series has not been used to explain selectivity in anion exchange chromatography. In Sections 5.2.1.2 and 5.2.1.3 the Hofmeister relationship to the anions and cations of interest in this study are presented. In Section 5.2.2 the most prevalent model explaining the Hofmeister series, the Collins-Washabaugh model, is presented. This model will be used in this chapter to explain our observations in ion pair chromatography.

5.2.1.2 Anions

There are several methods for the classification of the behaviour of the anions. Despite this the Hofmeister series remains virtually constant, regardless of the technique used. One of the primary techniques used to classify the anions is their retention times on a Sephadex G-10 (gel filtration) column. An example of such a separation was published by Washabaugh and Collins[28] depicting the order of retention of several anions:

$$\underset{\text{kosmotropic}}{SO_4^{2-} \approx HPO_4^{2-}} < F^- < Cl^- < Br^- < \underset{\text{chaotropic}}{I^- (\approx ClO_4^-)} < SCN^- \quad (5.1)$$

The classification of an anion as being kosmotropic or chaotropic is dependant upon the anion's influence on the hydrogen bonding structure of water. Those anions that are classified as kosmotropic are able to enhance the natural hydrogen bonding order of water. Chaotropic anions on the other hand are those anions that disrupt the hydrogen bonding in water. The order of elution from a gel filtration column (eq. 5.1) follows the increase in the chaotropic nature of the anion. As stated above, other methods of exploring ion behaviour display similar Hofmeister series[26, 27, 29]. In this chapter the test anions were chosen so as to span the Hofmeister series. These include chaotropic anions (iodide, thiocyanate and perchlorate) and kosmotropic anions (iodate, chloride and nitrite). There are also two anions (bromide and nitrate), which are intermediate between the kosmotropic and chaotropic anions.

A number of multiply charged anions (sulphate, thiosulphate and chromate) are included in my study. Of these, only sulphate has been previously classified in the Hofmeister series[22-24, 27, 30] (series 5.1). However, thiosulphate and chromate display similar retention behaviour to sulphate in ion chromatography.[31] Therefore it is assumed that thiosulphate and chromate are similarly kosmotropic.

5.2.1.2 Cations

It is known that anions have the greatest influence on the Hofmeister effect,[22, 23, 28] yet the effect of the cations cannot be fully discounted. Furthermore, the cation behaviour is particularly relevant in this study, as the change in the cationic IPA is what will lead to the alterations in the retention factors and selectivities. It is known that the different IPAs will have different interactions with the same anions. However little is known of the classification of the three IPAs in terms of their relative kosmotropic/chaotropic natures. Though there has been some mention in the literature of the increase in retention of chaotropic anions by phosphonium cations,[13, 14] little characterization of this effect has been published. For sulfonium even less is known. There are literally no publications that deal with sulfoniums cations as IPAs in chromatography. Therefore there was no prior knowledge as to how the sulfoniums would affect the retention of anions in ion pair chromatography. Ironically sulfoniums have actually been analyzed through a number of means[32, 33], including ion pair chromatography[34]. An understanding of the influences on ion retention in ion pair chromatography can help us understand the nature of these cations. This is particularly evident in the work of Aced *et al.*[34] wherein the separation of various onium compounds was performed using ion pair chromatography. They found that the phosphoniums ions were more strongly retained than the ammoniums ions under identical conditions. They did also separate sulfoniums ions. Unfortunately, these separations were performed under different conditions that those of phosphonium or ammonium, making any direct comparisons impossible.

Ideally knowing the placement of phosphonium, ammonium and sulfonium within the Hofmeister series would allow for a simple classification of their relative natures (chaotropic *versus* kosmotropic). Yet no such classification was found in a comprehensive search of the literature. As a result the relative nature of the IPAs has been determined from other reported experimental evidence. In particular the work of Wandlowski *et al.*[35] proves highly conclusive as to the relationship of ammonium and phosphonium with regard to the Hofmeister series. They determined the enthalpy change on ion transfer across an interface for equivalent ammonium and phosphonium salts. The removal of a chaotropic species from an aqueous environment has a negative change on the entropy, whereas the entropy change for the removal of a kosmotropic species would be positive[35]. The values obtained for tetramethylammonium and tetramethylphosphonium were -27.4 and -41.8 J mol^{-1} K^{-1} respectively. For comparison, the entropy change for perchlorate, a strong chaotrope (series 5.1), was -68.8 J mol^{-1} K^{-1}. Also the entropy change becomes more positive with longer alkyl chains for both ammonium and phosphonium, though the magnitude of the entropy change for phosphonium remained lower than that of ammonium in all cases. From this, it can be inferred that both ammonium and phosphonium species are chaotropic, and that phosphonium ions are more chaotropic than ammonium ions. Sulfoniums have not been studied in such detail in the literature, and as such cannot be compared to ammonium or phosphonium. In Section 5.4.1.2 I will infer the kosmotropic/chaotropic nature of sulfonium based upon the observations of the retention of anions spanning the Hofmeister series.

5.2.2 Collins-Washabaugh Model

The prevailing model to explain the Hofmeister series was developed by Collins and Washabaugh[23]. Their classic paper summarized more than 900 papers dealing with the Hofmeister series, and has itself been cited over 450 times. The Collins-Washabaugh model is based on the conceptualization of a series of three layers of water surrounding a solvated ion, as depicted in Figure 5.1.

Each layer of water influences the adjacent layers through hydrogen bonding. The layer closest to the ion, L_1, is influenced directly by the ion itself. The layer furthest from the ion, L_3, is influenced by the bulk solution properties. This layer may contain electrolyte from the bulk solution. The intermediate layer, L_2, is a transitional layer and is subject to the influences of both L_1 and L_3, as both layers compete for hydrogen bonding with the water in L_2. Electrolyte from the bulk solution is assumed to not be present in layers L_1 and L_2.

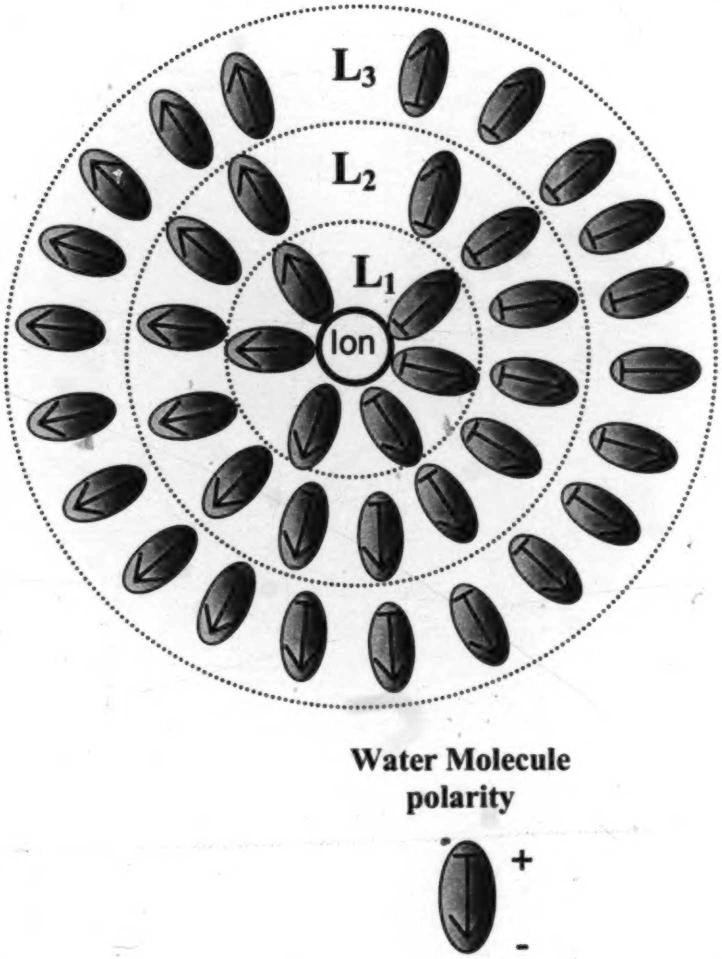

L3
L2
L1
Ion
Water Molecule
polarity
+
-

Figure 5.1 Schematic of solvated ion based upon the Collins & Washabaugh[23] model involving three layers solvating water (bulk solution excluded).

The solubility of an ion is determined by both its interactions with the waters surrounding it (L_1 layer) and the influences of the bulk solution on the water surrounding the ion (L_3 layer). Anions such as iodate, chloride and nitrite are kosmotropic. That is, these anions increase the structure of water, through the promotion of hydrogen bonds upon solvation[22, 23]. This leads to strong interactions with the L_1 waters that are carried over to the L_2 layer. This increase in water structure causes these ions to be highly soluble in water. The multiply-charged anions (sulphate, thiosulphate, and chromate) are also kosmotropic (Sec. 5.2.1), and so would be expected to behave similarly.

Conversely the polarizable anions (*e.g.*, iodide, thiocyanate, and perchlorate) are chaotropic. Chaotropic ions decrease the structure of water around them. As a result there are only very weak interactions between the anion and the water molecules in the L_1 layer. By not promoting structured hydrogen bonding in the L_1 waters, the influence of L_1 on the structure of L_2 water is minimal. This in turn leads to the structure of the water in L_2 being predominantly determined by the nature of the L_3 hydrogen bonding. Since the chaotropic anions do not promote hydrogen bonding with the surrounding water, they are less soluble.

Intermediate anions such as bromide and nitrate fall between the two previous classes of anions. They neither promote hydrogen bonding with water as much as the kosmotropics, nor do they decrease the structure as much as the chaotropics.

5.2.3 Hofmeister Series and Ion Chromatography

The Hofmeister series has only been used a handful of times to understand the elution order seen in anion exchange chromatography[24, 27, 29, 36, 37]. Even then, these papers did not delve into the aspects of the series that lead to the resultant retention order. My work attempts to fill this void and presents a proposal for how the Collins and Washabaugh model[23] of the Hofmeister series influences the retention of ions in ion pair chromatography.

A variant of ion chromatography known as electrostatic ion chromatography uses a zwitterionic stationary phase[38-40]. A typical ranking of retention of common anions through electrostatic ion chromatography is[41]:

$$SO_4^{2-} < Cl^- < NO_2^- < CNO^- < Br^- < NO_3^- << ClO_3^- << I^- << SCN^- << ClO_4^- \quad (5.2)$$

This ranking coincides well with that seen for the Hofmeister series (5.1), with the exception that perchlorate is ranked as the most retained by electrostatic ion chromatography.

Retention in anion exchange chromatography, where the stationary phase has cationic sites for ion exchange, is firstly dependent upon electrostatic attraction. Thus, divalent anions such as sulphate are more strongly retained than monovalent anions such as chloride, and do not follow the expected Hofmeister series. Yet the monovalent anions do follow the Hofmeister series, with the most kosmotropic eluting first and the most chaotropic being the most retained. The standard retention order for a Dionex anion exchange column is[42]:

$$Cl^- < NO_2^- < Br^- < NO_3^- < SO_4^{2-} < I^- < CrO_4^{2-} < SCN^- \quad (5.3)$$

The similarity in orders arises from the requirement of having to strip the water from around an anion to interact with the stationary phase in ion chromatography[23]. This requires the stripping of the L_1 waters from around the anion and making them part of the bulk water. Therefore the greater the strength of the interactions of an anion with the L_1 water the less likely it will be for there to be significant interactions with the IPA.

In recent years there have been numerous modifications to the stationary phases used in anion exchange chromatography. The modifications pertain to the exchange sites. For instance, increasing the hydrophobicity of the exchanger can be achieved through an increase of the size of the alkyl chains on the cation. This leads to a reduction in retention of multivalent anions, while increasing retention for the monovalent anions, particularly for chaotropic anions[41]. Alternatively the exchange site can be made more hydrophilic through the incorporation of more hydrophilic species, such as alkanolamines, onto the cation site. Such hydrophilic exchangers are also referred to as *hydroxide selective* exchange sites, due to the increased effectiveness of hydroxide eluents with these exchange sites. Hydrophilic modified anion exchangers are favoured for the separation of chaotropic anions as the kosmotropic character of the cationic exchanger reduces the retention of the chaotropic anions[41].

However while the tailoring of the ion exchangers has been successful, the alterations appear to have been pragmatically driven - without a solid fundamental basis. In particular there is no suggestion that the importance of the Hofmeister series has been recognized. Obviously, the alterations impact the structure of the water around the exchange site. Increasing the structure of water when the exchange site is hydrophilic, and decreasing the structure when hydrophobic. As will be shown in Sections 5.4.1 the changes to the structure or water can favour or disfavour interactions between ions. The same principles that are applied in the theory for ion pair chromatography can be applied to the ion exchange sites and the resultant changes in retention that are seen.

The selectivity towards certain anions seen by ion exchange stationary sites is also possible by IPAs. The IPA is capable of similar influence on the structure of the water molecules surrounding it. In addition the ions used as IPAs are capable of penetrating the L_3 region around an analyte anion and are capable of effecting the hydrogen bonding in that region[23]. The extent of this influence will be based on the kosmotropic or chaotropic nature of the IPA molecule. Alteration of the water structure in L_3 impacts the structure of L_2. As always the hydrogen bonding of L_2 would be determined by the strength of the influences of L_1 and L_3, with the layer of greatest structure determining the hydrogen bonding arrangement of L_2. However changes in the nature and amount of the IPA will influence this structure, and thereby alter the retention characteristics. The degree of these changes will be influenced both by the nature of the IPA and the anion of interest. Our study incorporates a number of different anions as well as distinct IPAs at various concentrations and eluent conditions to help identify the Hofmeister influences on the separation.

5.3 Experimental

5.3.1 Apparatus

All separations were performed with a Metrohm (Metrohm, Herisau, Switzerland) separation system, consisting of: 733 IC Separation Center; MSM suppressor; 752 Pump Unit; 762 IC Interface; and 709 IC Pump. All sample injections were done with a 20 µl sample loop. Data acquisition was performed with a combination of a Metrohm 762 data acquisition system and a Pentium II processor with IC Net 2.1 software (Metrohm). Data acquisition was set at 30 Hz. Conductivity measurements were obtained with a Dionex ED-50A electrochemical detector (Dionex, Sunnyvale, CA, USA).

All separations were performed on a 2.1 mm i.d. x 100 mm Xterra MS C_{18} column packed with 3.5 µm particles (Waters Corporation, Milford, MA, USA). The Xterra columns are unique in that they are a hybrid particle. The silica used to form the stationary phase support is composed of both inorganic (SiO_2) and organic ($CH_3SiO_{1.5}$) silica. The advantage of this is that it incorporates the characteristics of both the traditional support and polymeric support. According to the manufacturer the resulting particles offer improved efficiency for basic analytes and greater stability at high pH, while otherwise maintaining the expected characteristic of a reversed phase C_{18} column. The column was maintained at a constant temperature of 30 ±0.1 ° C using an Eppendorf CH-30 column heater (Alltech, Deerfield, IL, USA) and TC-50 (Alltech) temperature control unit. All tubing between the injector and detector was 0.005" i.d. PEEK (Upchurch Scientific, Oak Harbor, WA, USA).

5.3.2 Reagents

All aqueous solutions were prepared using distilled-deionised water (Nanopure Water Systems, Barnstead, Chicago, IL, USA). The lowest grade of chemicals used was reagent grade, with higher grades used as available. All solutions were stored in Nalgene containers (Nalgen Nunc International, Rochester, NY, USA). Stock analyte solutions were prepared to ~ 10^{-2} M and stored in the dark until diluted with distilled-deionised water to the desired concentrations the day of analysis. Potassium salts of: chloride (EM Scientific, Darmstadt, Germany); iodate (ACP Chemicals Inc., Montreal, QC, Canada); thiocyanate, bromide and chromate (Fisher, Nepean, ON, Canada) were used as standards. Additional standards of sodium salts were: nitrite, iodide and sulphate (BDH Inc., Toronto, ON, Canada); nitrate (ACP Chemicals Inc., Montreal, QC, Canada); perchlorate and thiosulphate (Anachemia, Montreal, QC, Canada). Suppressor regenerant solutions were prepared (~ 10^{-2} M) with sulphuric acid from Anachemia and HPLC grade methanol (Fisher). Suppressor rinse solutions were prepared with 15% methanol in nanopure water. Reagents for the sulfonium synthesis were: perchloric acid (70%); diethyl-ether and 1-butanol (Fisher); and butyl sulphide (Aldrich Milwaukee, WI, USA). Anion exchange resin, AG 2-X8 100-200 mesh chloride form, was obtained from BioRad Laboratories (Richmond, CA, USA) and was converted to the bicarbonate form with sodium bicarbonate (EM Scientific). The column contained approximately 80 ml of resin (1.4 meq/ml) and required approximately 1.5 liters of 1 molar sodium bicarbonate for complete conversion. Complete conversion was confirmed by the absence of precipitate upon addition of silver chloride (analytical reagent, BDH Inc.) in effluent acidified with an excess of reagent grade nitric acid (Anachemia). The nitric acid was used to convert the bicarbonate to carbon dioxide such that it would not interfere with the precipitation test for chloride, the original anionic form of the resin. The IPAs tetrabutylphosphonium bromide (98%) and tetrabutylammonium bromide (99%) were purchased from Aldrich. Vacuum filtration of all eluents and of sulfonium precipitate was performed with nylon (0.22 μm pores) filter papers from Fisher.

5.3.3 Sulfonium Synthesis

Some sulfoniums are commercially available, such as triphenylsulphonium hexafluoroaresenate from Alfa Aesar (Ward Hill, MA, USA). However none of the commercially available sulfoniums have a structure similar to the ammonium and phosphonium IPAs used in ion pair chromatography. Most commercially available sulfoniums contain at least one phenyl substituent, which makes them unsuitable for ion pair chromatography if UV absorbance detection is used. Therefore tributylsulphonium perchlorate, the closest analog to the tetrabutyl forms of ammonium and phosphonium was synthesized using a scaled up version of the procedure of Milligan and Minor[43].

$$\text{R-S-R} + \text{R-OH} + HClO_4 + \text{heat} \rightarrow R_3\text{-}S^+ClO_4^- + H_2O \qquad (5.4)$$

$$R = CH_3(CH_2)_3$$

The original synthesis requires the reaction of 5 mmoles of butylsulphide (dibutylsulphide) in 5 ml of 1-butanol with 5 mmoles of 70% perchloric acid. This reaction mixture is refluxed for 24 hours before it is cooled to room temperature and diluted in 50 ml of ether. The precipitate, tributylsulphonium perchlorate, was filtered and collected. This procedure would produce about a 50% yield (~0.75 g) of tributylsulphonium, which is in agreement with the reported yields[43]. However for the quantities of TBS needed the synthesis was increased in scale for faster production of the salt. It was found that an increase of all reagents by a factor of 4 would provide a yield of ~65% (~4 g) of sulfonium for a reaction of about 40 hours. In addition it was found that an 8 fold increase in reagents was able to yield as much as a 75% yield (9 g) of sulfonium after over 60 hours of refluxing. No difference was noted in the quality of the products recovered from any of the different reaction conditions.

The product from the reaction was confirmed through both elemental analysis and nuclear magnetic resonance. The elemental analysis was: 12% chloride, 10% sulphur, 47% carbon and 9% hydrogen. Showing that the percent composition of the elements of the product matches the expected values to within 1%. The proton NMR of the product, dissolved in deuterated methanol (Aldrich), shows a clean spectra with the chemical shifts and peak multiplicity consistent with the literature values for tributylsulphonium perchlorate.

5.3.4 Eluent Preparation

All eluents consisted of an IPA, methanol and sodium bicarbonate, at pH 7.5. The IPAs were converted to their respective bicarbonate salts before preparation of the eluents using a 30 cm x 2 cm i.d. column packed with AG 2-X8 100-200 mesh chloride-form anion exchange resin. The resin was converted to the bicarbonate form as previously described (Section 5.2.2), then thoroughly rinsed with distilled-deionised water. Solutions of the IPAs of about twice the final eluent concentration were passed through the ion exchange column. This allows the rinsing of the column with water to ensure quantitative elution of the IPAs. This was possible for both tetrabutylammonium bromide and tetrabutylphosphonium bromide which were readily soluble in water; solubility in water was achieved as high as 150 mM for both IPAs. The column was run at approximately 5 ml/min, and 250-500 ml of effluent was collected. The effluent then containing the IPA in the bicarbonate form was used to prepare the eluents for ion pair chromatography.

The solubility of the tributylsulphonium perchlorate was limited in water (~ 20 mM). In order to efficiently achieve the desired concentration of a stock solution to prepare the eluents the sulphonium was dissolved in methanol. Though it had only slightly better solubility in methanol (~30 mM) the ability to easily evaporate the methanol to the desired concentration was an advantage over water. The low concentration TBS-methanol solution was passed through the ion exchange column, which was tested and found to be suitable for use with methanol. The collected effluent was then evaporated to make a 250 mM solution of TBP chloride, which has a much higher solubility than the perchlorate salt. This solution was used in the preparation of eluent for the IPA concentration study, Section 5.4. Solutions were prepared from the required amount of TBS-methanol to have the proper concentration in the eluent. Additional methanol was added to bring the total volume percentage to 30%.

For the methanol study a more efficient procedure was sought, one that would allow easy alteration of the methanol content without altering the TBS concentration. As a consequence an alternate procedure was developed using precipitation reactions. Earlier work with tetrabutylphosphonium had shown it to be highly insoluble with perchlorate in water. Therefore solid tetrabutylphosphonium bromide was added to the solution of tributylsulphonium to precipitate the perchlorate anion. For these precipitations a slight excess of tetrabutylphosphonium bromide was used to ensure the full precipitation of the perchlorate, which was removed by filtration. The excess tetrabutylphosphonium bromide was removed by subsequent precipitation by the addition of an excess of sodium perchlorate, and the solution was again filtered. The small amount of perchlorate left in the tributylsulphonium was removed by anion exchange column (the same column as used for the other IPAs) used to convert the tributylsuphonium bromide into the bicarbonate form. In the same process the sodium is converted to the bicarbonate form to act as the buffer for the eluent.

Qualitative mass spectrometry results confirmed the presence of tetrabutylphosphonium in the solution after filtration. Yet a rough mass balance of the precipitate reveals that at most there is 4% TBP in the TBS solution. Though this may not be insignificant it is clear from the differences seen in the methanol studies Section 5.5 where the TBS and TBP solutions showed significant differences.

5.3.5 Procedure

The eluents used for these studies were all prepared in the same manner. To a concentrated stock of the IPA of interest a measured volume of methanol was added to achieve the desired volume-to-volume percentage. Then the required amount of sodium bicarbonate was dissolved and the solution was brought to about 90% of the final desired volume. The pH of the solution was measured and adjusted with phosphoric acid. The eluent was then diluted to volume, vacuum filtered and stored in Nalgene containers.

All separations were performed at 30 °C at a flow rate of 0.2 ml/min. The column was equilibrated with each new eluent for at least 1 hour (equivalent to 30 column volumes). When the eluent was changed the column was rinsed with 100% methanol at 0.3 ml/min for at least 1 hour (45 column volumes), and then the next eluent equilibration was started. Column volumes were based upon the manufacturer's data, placing the 2.1 x 100 mm column at a volume of 0.4 ml.

5.4 Results and Discussion

5.4.1 Observed Retention Behaviour with the Various IPAs

The IPAs will first be characterized by investigating the effect of IPA concentration on ion retention. The eluents were prepared as described in Section 5.3.4. The percent methanol and concentration of sodium bicarbonate were constant at 30% and 20 mM respectively, and the pH of the eluents was 7.5 (Section 5.3.5). The only variable in the eluent composition was the type and concentration of the IPA. Eluents were prepared with 15 mM-75 mM of each IPA. This range was chosen based on the guidelines established by Bartha *et al.*[16]

to ensure that the IPA does not form micelles. The formation of micelles in the eluent would result in micellar liquid chromatography separations, which has significantly different separation characteristics to ion pair chromatography[44]. If micelles were present in the eluent, a significant decrease in the retention factor of all analytes would occur, when compared to lower IPA concentrations[45]. Retention factors observed for the test analytes with tetrabutylammonium (TBA), tetrabutylphosphonium (TBP) and tributylsulphonium (TBS) cations are tabulated in Tables 5.1, 5.2 and 5.3, respectively. The predicted behaviour of micellar liquid chromatography was not observed. Thus it is clear that the concentration of the IPAs did not reach their critical micelle concentrations. Due to the low retention observed with TBS (Table 5.3), a more limited set of test anions were used in its characterization.

5.4.1.1 Kosmotropic Monovalent Anions

The retention behaviour of iodate, chloride, nitrite from Tables 5.1-5.3 is plotted in Figures 5.2, 5.3 and 5.4, respectively. A first observation is that the retention of these anions is essentially independent of the concentration of IPA. This can clearly be seen in the overall change in retention factor over the range studied. For chloride the total change in k' was 0.01 for TBA, 0.02 for TBP and 0.01 for TBS. However though there was little change within the retention factors over the range studied, there were differences in the retention factors between the different IPAs. For instance, at 60 mM IPA, the k' for chloride is 0.21 with TBA, 0.30 with TBP and 0.19 with TBS. Further examination of Tables 5.1 and 5.2

Table 5.1 Retention factors for various concentrations of tetrabutylammonium bicarbonate[a]

	Retention Factor (One Standard Deviation)				
	15mM	**30mM**	**45mM**	**60mM**	**75mM**
Iodate	0.150 (0.006)	0.144 (0.005)	0.176 (0.027)	0.169 (0.022)	0.170 (0.021)
Chloride	0.210 (0.009)	0.224 (0.011)	0.207 (0.008)	0.214 (0.006)	0.201 (0.005)
Nitrite	0.218 (0.002)	0.253 (0.002)	0.253 (0.006)	0.229 (0.009)	0.222 (0.005)
Bromide	0.307 (0.017)	0.357 (0.017)	0.360 (0.027)	0.368 (0.017)	0.367 (0.022)
Nitrate	0.378 (0.012)	0.421 (0.018)	0.450 (0.018)	0.481 (0.010)	0.466 (0.007)
Sulfate	0.471 (0.019)	0.455 (0.014)	0.404 (0.024)	0.397 (0.018)	0.356 (0.016)
Chromate	0.529 (0.016)	0.550 (0.029)	0.489 (0.050)	0.497 (0.022)	0.486 (0.029)
Thiosulfate	0.559 (0.019)	0.624 (0.027)	0.594 (0.026)	0.572 (0.022)	0.534 (0.026)
Iodide	0.713 (0.024)	0.746 (0.028)	1.044 (0.036)	1.112 (0.033)	1.144 (0.041)
Thiocyanate	1.592 (0.006)	2.092 (0.040)	2.410 (0.064)	2.769 (0.094)	2.712 (0.053)
Perchlorate	1.645 (0.027)	2.425 (0.043)	2.913 (0.054)	3.313 (0.049)	3.505 (0.045)

a. Elution conditions: 30% methanol, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 µm), flow 0.3 ml/min

Table 5.2 Retention factors for various concentrations of tetrabutylphosphonium bicarbonate[a]

	Retention Factor (One Standard Deviation)				
	15mM	30mM	45mM	60mM	75mM
Iodate	0.205 (0.009)	0.178 (0.007)	0.165 (0.005)	0.153 (0.004)	0.165 (0.004)
Chloride	0.318 (0.023)	0.311 (0.019)	0.335 (0.026)	0.300 (0.007)	0.298 (0.009)
Nitrite	-	-	0.413 (0.008)	0.364 (0.035)	-
Bromide	0.502 (0.018)	0.567 (0.020)	0.601 (0.021)	0.601 (0.020)	0.630 (0.023)
Nitrate	0.576 (0.036)	0.689 (0.025)	0.770 (0.033)	0.767 (0.009)	0.773 (0.011)
Sulfate	0.801 (0.051)	0.682 (0.037)	0.677 (0.026)	0.582 (0.028)	0.513 (0.007)
Chromate	0.961 (0.062)	0.955 (0.029)	0.957 (0.029)	0.795 (0.049)	0.834 (0.022)
Thiosulfate	1.033 (0.031)	1.059 (0.028)	1.057 (0.026)	0.970 (0.026)	0.946 (0.029)
Iodide	1.344 (0.030)	1.873 (0.051)	2.133 (0.045)	2.209 (0.051)	2.385 (0.060)
Thiocyanate	3.038 (0.204)	4.271 (0.183)	4.903 (0.142)	4.915 (0.132)	5.279 (0.042)
Perchlorate	2.944 (0.355)	5.290 (0.124)	6.676 (0.204)	6.924 (0.047)	-

a. Elution conditions: 30% methanol, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 μm), flow 0.3 ml/min

Table 5.3 Retention factors for various concentrations of tributylsulfonium bicarbonate[a]

	Retention Factor (One Standard Deviation)			
	30mM	**45mM**	**60mM**	**75mM**
Chloride	0.176 (0.001)	0.202 (0.010)	0.194 (0.011)	0.186 (0.028)
Sulfate	0.362 (0.034)	0.396 (0.032)	0.390 (0.028)	0.354 (0.027)
Thiosulfate	0.520 (0.089)	0.647 (0.027)	0.689 (0.020)	0.655 (0.045)
Iodide	0.542 (0.015)	0.664 (0.038)	0.733 (0.033)	0.744 (0.036)
Thiocyanate	-	1.316 (0.045)	1.512 (0.040)	1.546 (0.061)
Perchlorate	1.020 (0.028)	1.364 (0.031)	1.554 (0.036)	1.672 (0.042)

a. Elution conditions: 30% methanol, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 μm), flow 0.3 ml/min

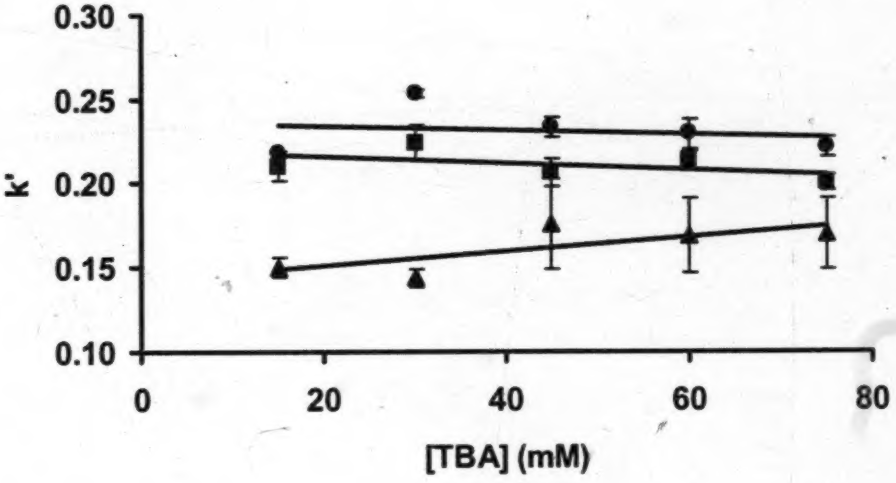


Figure 5.2 Retention factors of iodate (▲), chloride (■) and nitrite (●) versus TBA concentration. Error bars represent one standard deviation. **Elution conditions:** 30% methanol, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 μm), flow 0.3 ml/min

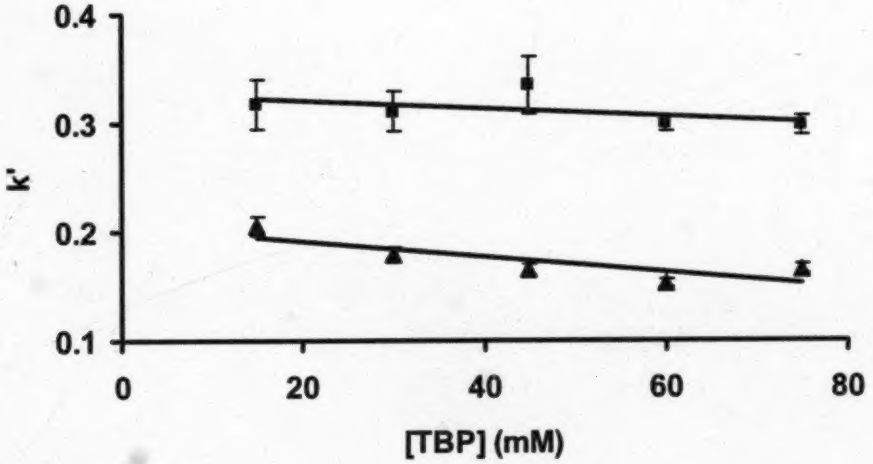


Figure 5.3 Retention factors of iodate (▲) and chloride (■) versus TBP concentration. Error bars represent one standard deviation. **Elution conditions:** 30% methanol, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 µm), flow 0.3 ml/min

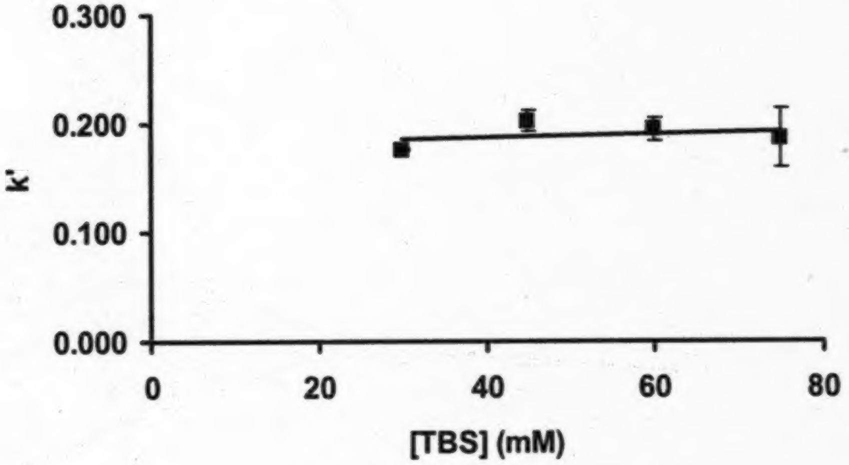


Figure 5.4 Retention factors of chloride (■) versus TBS concentration. Error bars represent one standard deviation. **Elution conditions:** 30% methanol, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 µm), flow 0.3 ml/min

shows that retention of the kosmotropic monovalent anions is 30% greater with TBP than TBA. The low retention observed with TBS limited the study of kosmotropic anions. Regardless, the k' for chloride was slightly lower for TBS than TBA for all conditions studied. Thus, the general retention trend observed is:

$$\text{TBS} \leq \text{TBA} < \text{TBP} \quad (5.5)$$

5.4.1.2 Chaotropic Monovalent Anions

A comparison of the data in Tables 5.1-5.3 and Figures 5.5-5.7, shows that the retention of chaotropic anions (iodide, thiocyanate, perchlorate) is much greater than that of the kosmotropic anions. For instance, with 45 mM TBA chloride has a k' of 0.21, whereas perchlorate is 2.91. This is consistent with the behaviour in ion chromatography[41]. Also, unlike the kosmotropic monovalent anions, retention of the chaotropic anions increases dramatically with increasing IPA concentration. For instance, retention of perchlorate more than doubled when the TBA concentration was increased from 15 to 75 mM, where chloride showed no change in retention (Table 5.2).

The difference between the IPAs is evident in the magnitude of change in the retention factors. When k' is plotted versus the logarithm of the IPA concentration a near linear trend is seen. There is also a distinct difference in the slopes of the lines achieved for each of the chaotropic anions. For all the IPAs the lowest slope was seen for iodide, with values of 0.52 for TBS, 0.62 for TBA and 1.47 for TBP. This trend was also seen for the anion with the greatest slope,

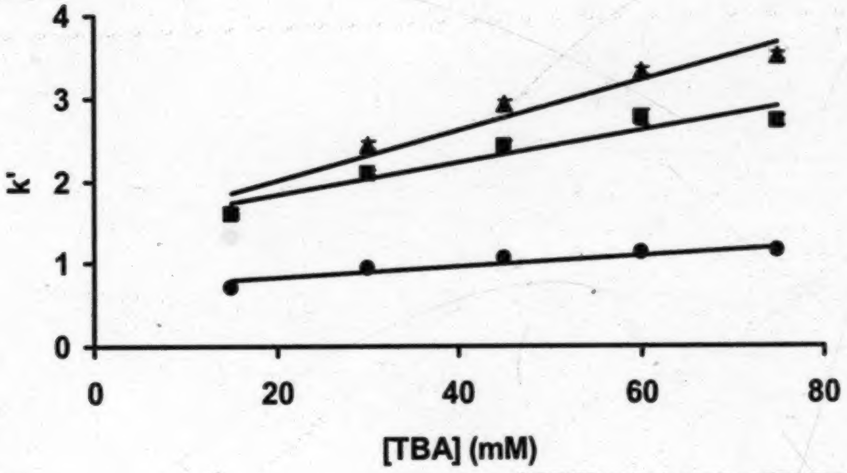


Figure 5.5 Retention factors of iodide (●), thiocyanate (■) and perchlorate (▲) versus TBA concentration. Error bars represent one standard deviation. **Elution conditions:** 30% methanol, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 µm), flow 0.3 ml/min

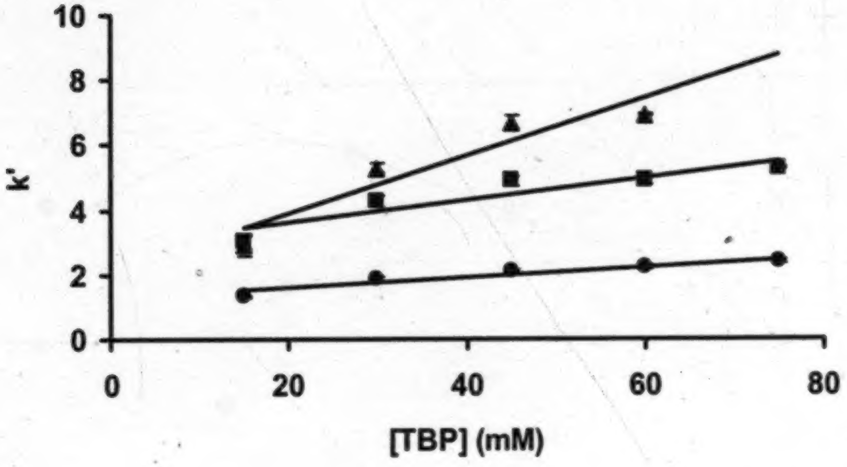


Figure 5.6 Retention factors of iodide (●), thiocyanate (■) and perchlorate (▲) versus TBP concentration. Error bars represent one standard deviation. **Elution conditions:** 30% methanol, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 µm), flow 0.3 ml/min

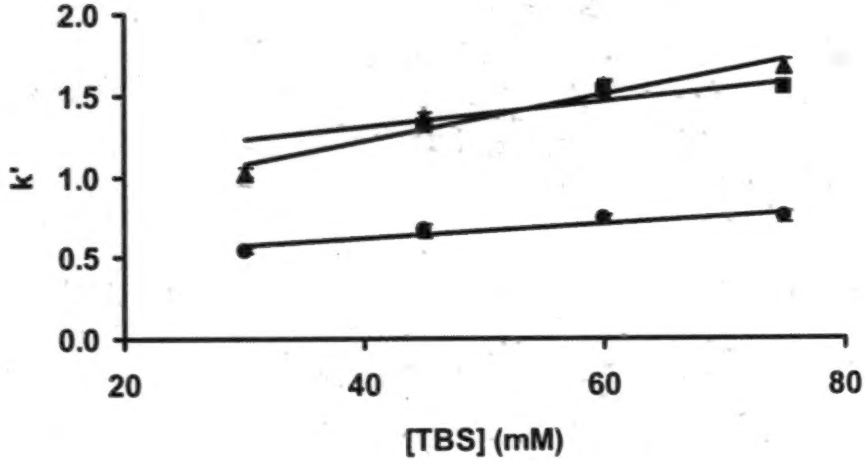


Figure 5.7 Retention factors of iodide (●), thiocyanate (■) and perchlorate (▲) versus TBS concentration. Error bars represent one standard deviation. **Elution conditions:** 30% methanol, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 µm), flow 0.3 ml/min

perchlorate. The slope for the plot of perchlorate was 1.64 with TBS, 2.69 with TBA and 5.51 with TBP. As can be seen in Figures 5.5, 5.6 and 5.7 the magnitude of the increasing retention factor for all the chaotropic anions is greatest for TBP and least for TBS.

The work of Wandlowski *et al.*[35] showed the phosphoniums ions have greater chaotropic character than ammoniums ions. As can be seen in my work this conclusion appears to be representative of the influence of the IPA on the retention factor of the chaotropic anions. Thus by comparing the slopes for the same anion we can classify the chaotropic character of the IPAs. Consequently we can now accurately claim that TBS is the least chaotropic of the IPAs examined.

5.4.1.3 Intermediate Monovalent Anions

The intermediate anions (bromide, nitrate) are also affected by changing the IPA concentration, as shown in Tables 5.1 and 5.2 and Figures 5.8 and 5.9. The intermediate anions are retained more than the kosmotropic anions. This is consistent with the behaviour seen in ion chromatography[41]. The intermediate anions are transitional in their characteristics towards the structure of water. That is there is no binary change from kosmotropic to chaotropic[23]. As a result the intermediate anions do not act exactly like either of the two classifications. They show a significantly lower retention than chaotropic anions (e.g., perchlorate, thiocyanate), and elute before the doubly charged species under most ion pair chromatographic conditions, similar to the kosmotropic anions. Yet as was seen with the chaotropic anions (Sec. 5.4.1.2), the intermediate anions experience an

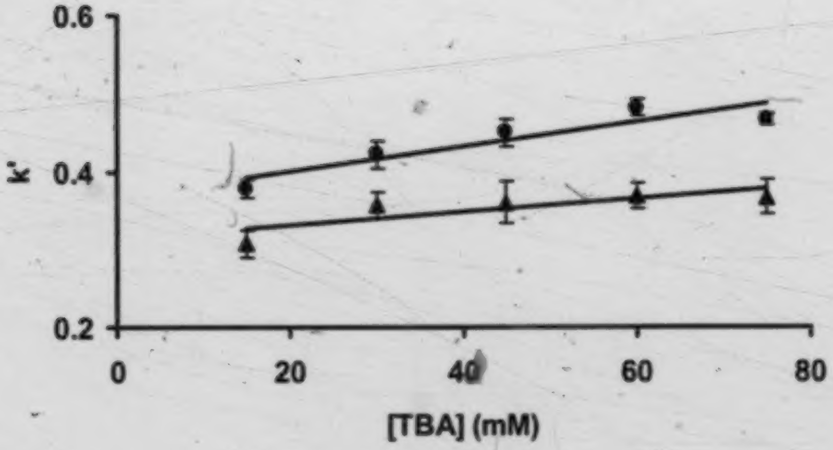


Figure 5.8 Retention factors of nitrate (●) and bromide (▲) versus TBA concentration. Error bars represent one standard deviation. **Elution conditions:** 30% methanol, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 µm), flow 0.3 ml/min

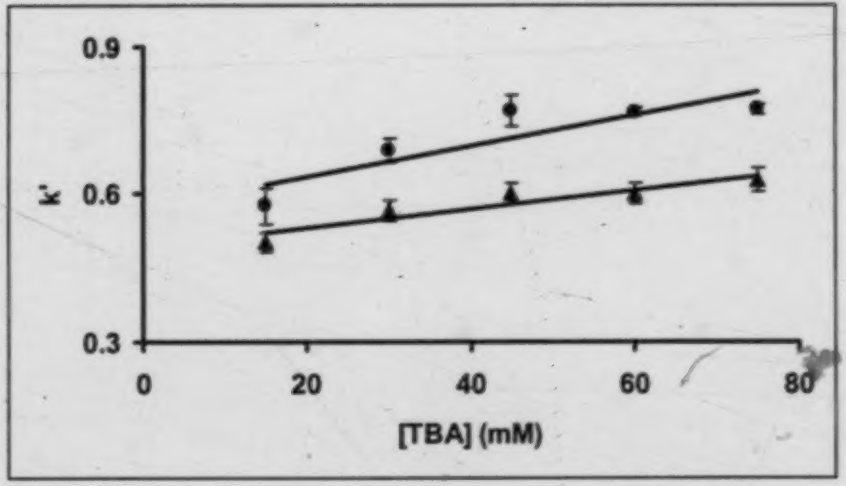


Figure 5.9 Retention factors of nitrate (●) and bromide (▲) versus TBP concentration. Error bars represent one standard deviation. **Elution conditions:** 30% methanol, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 µm), flow 0.3 ml/min

increase in retention factor with increasing IPA concentration. In Figures 5.8 and 5.9 the slopes for the intermediate anions are lower than those seen for the chaotropic anions (Fig. 5.4-5.6). Bromide has a slope of 0.08 with TBA and 0.18 with TBP. Similarly nitrate has a slope of 0.14 with TBA and 0.29 with TBP. This reflects the positioning of the anions on the Hofmeister series, demonstrating a moderated influence of the change in concentration of the IPA.

5.4.1.4 Multivalent Anions

The retention behaviour of the multivalent anions (sulphate, thiosulphate and chromate) is presented in Tables 5.1, 5.2 and 5.3 and plotted in Figures 5.10, 5.11 and 5.12. The multivalent anions, unlike the rest of the Hofmeister series, do not seem to follow the trends that are anticipated from ion chromatography. This is particularly evident in sulphate (a kosmotropic anion) eluting after the intermediate anions[23, 31, 41, 46]. Though the multiply charged anions are considered to be kosmotropic, they have greater retention than the seemingly less kosmotropic (based on Hofmeister series) singly charged anions (chloride, nitrite, and iodate). This increased retention is not surprising however since the basis for the Hofmeister series focuses on solubility rather than ionic interactions[22, 23, 28]. Given that the retention mechanism in ion pair chromatography is primarily ionic interactions it is not surprising that the multiply charged anions would have the retention factors seen in our work. However the multiply charged anions do exhibit a unique change in retention when the concentration of the IPA in the eluent is increased. When the concentrations of either TBA or TBP are increased the overall effect on the k' of the multivalent anions is a decrease in retention. In

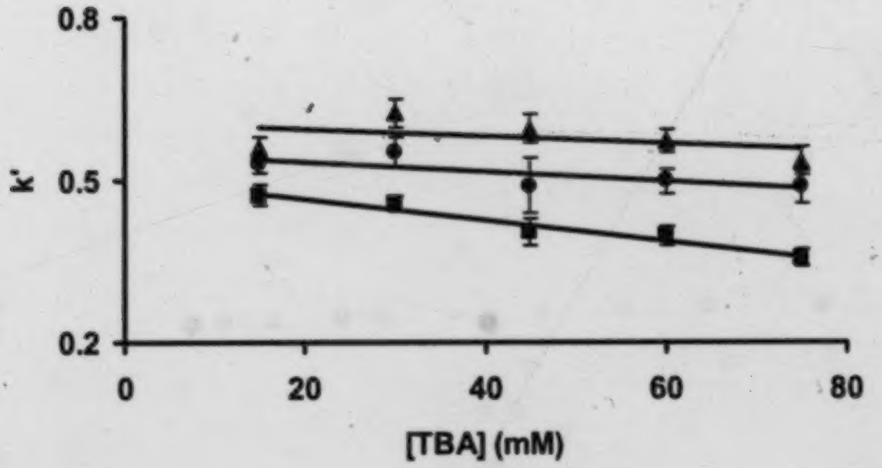


Figure 5.10 Retention factors of sulphate (■), chromate (●) and thiosulphate (▲) versus TBA concentration. Error bars represent one standard deviation.

Elution conditions: 30% methanol, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 µm), flow 0.3 ml/min

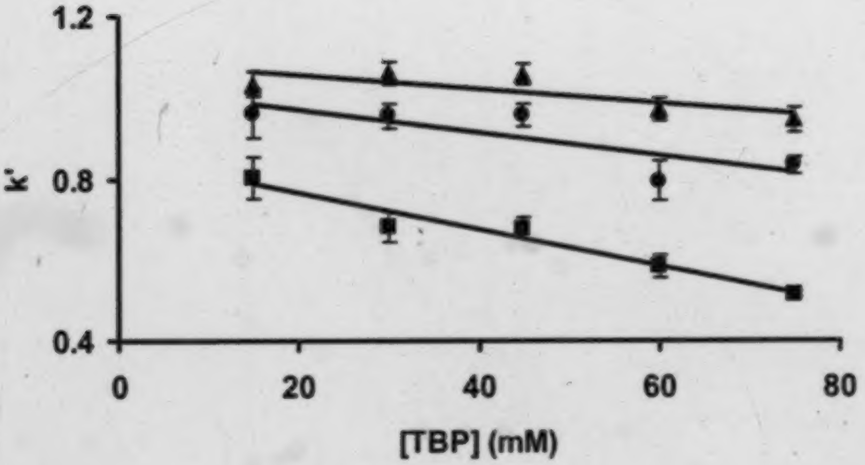


Figure 5.11 Retention factors of sulphate (■), chromate (●) and thiosulphate (▲) versus TBP concentration. Error bars represent one standard deviation.

Elution conditions: 30% methanol, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 µm), flow 0.3 ml/min

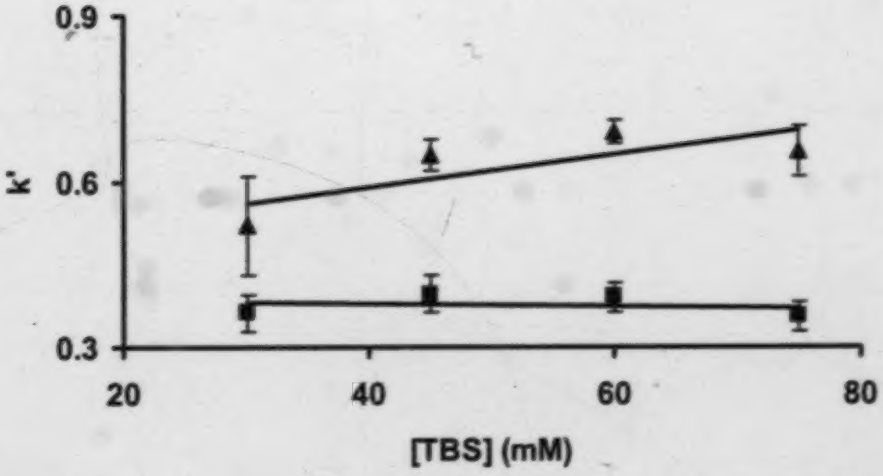


Figure 5.12 Retention factors of sulphate (■) and thiosulphate (▲) versus TBS concentration. Error bars represent one standard deviation. **Elution conditions:** 30% methanol, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 µm), flow 0.3 ml/min

Figures 5.10-5.12, the behaviour is rectilinear after a brief increase in k' at low IPA concentration. Though the linear correlation is not perfect, it is clear that k' decreases, and that these decreases depend on both the nature of the anion and IPA. With TBA the slope of the linear correlation was -0.04 for thiosulphate, -0.05 for chromate and -0.16 for sulphate. However with TBP the slopes were much more severe at -0.11 for thiosulphate, -0.17 for chromate and -0.43 for sulphate. Once again this reveals a trend that can be correlated to the chaotropic nature of the IPA. From this data it would be anticipated that the decrease in k' would be even less for TBS. Yet the results are quite different from what would be anticipated. Figure 5.12 reveals that the k' for thiosulphate with TBS increases from 0.52 at 30 mM to 0.69 at 60 mM, having a slope of 0.36. Whereas the k' for sulphate remains centered on 0.38 for the range of IPA concentrations studied. This is significantly different than the behaviour in Figures 5.10 and 5.11. A more detailed explanation for these differences will follow in Section 5.4.2.4.

5.4.2 Mechanism of IPA Influence on Retention

It is clear from the data above that there is a difference in the retention characteristics of the three IPAs that I have investigated. The three IPAs and the changes in their concentration, vary in their influence on different classes of anions. Below I propose a mechanism through which these changes occur.

The proposed mechanism is based upon the double-layer theory of ion pair chromatography. An overview of this theory can be found in Section 1.3.3, and a more detailed look at the model and its variations has been published by Chen *et* *al.*[47]. For the first time however the Hofmeister influence of both the anions and cations will be brought into the discussion.

Within the double-layer theory the retention of an analyte is dependant upon its proximity to the Outer Helmholtz Plane (OHP). This is due to the decay of the potential due to the IPA adsorbed within the Inner Helmholtz Plane (IHP). The closer an analyte ion can approach the surface the greater retention it experiences. The incorporation of the Hofmeister influence of the ions is the result of the impact of the hydration of ions on their ability to approach a double-layer surface. An extensive study of the effect of hydration of ions and their interactions with charged interfaces was undertaken by James and Healy[48-50]. Though this work investigated the double-layer from the perspective of surface chemistry it is based upon the Gouy-Chapman model, the same used for ion pair chromatography. Of the many discoveries from these papers the most important for my work was the revelation that a primary barrier for the approach of an ion to a surface is the ion-solvent interactions[50]. Of particular interest are the solvent molecules which are in close proximity with the ion. The water present around the ion limits the distance within which the ion can approach the charged surface (IHP). Thus the solute acts as a key influence to the retention which can be achieved by an anion under any given conditions. The incorporation of the Collins and Washabaugh model into the Hofmeister effect[23] becomes an important tool in understanding the results that I obtained. The IPA will alter the influence of the bulk solution on the hydrogen bonding structure of the water in the layers surrounding the analyte ion. An increase in the hydrogen bonding structure around the ion will decrease the depth of penetration that the ion can achieve in the double-layer. Conversely a decrease in the hydrogen bonding structure around the ion will allow for greater penetration of the double-layer, leading to greater retention of the analyte.

5.4.2.1 Influences on Kosmotropic Anions

As was shown in Section 5.4.1.1 the retention of the monovalent kosmotropic anions was independent of IPA concentration. Some literature has reported an increase in the retention factor of an analyte with increasing IPA concentration [16, 17, 51]. Conversely there have been reports of the retention factor being constant at different IPA concentrations[17, 51]. The potential for k' being independent of IPA concentration is that the stationary phase is saturated with IPA, even at the lowest IPA concentration. However, Bartha and Vigh[17] explicitly determined the surface concentration of IPA. They observed constant retention factors for adrenaline despite increasing surface concentration of the IPA. However they were unable to provide a definitive rational for their observations.

The monovalent anions iodate, chloride and nitrite have a strong kosmotropic characteristic. As such, a strong hydrogen bonding structure is formed within the L_1 water Figure 5.1. This in turn influences the structure of the waters in L_2, based on the Hofmeister series[23, 31, 41]. Increases in the IPA concentration cannot have a direct impact on the energy required to strip the L_1 waters from an anion as they cannot penetrate any deeper than the L_3 region of waters[23]. It is expected that the presence of increased amounts of an IPA will decrease the hydrogen bonding structure in L_3[35]. As the hydrogen bonding in L_3 diminishes the stability of the water in L_1 and L_2 will remain constant. As a result there should be little to no change in the energy required to remove the outer layers of water from the anions. Consequently the degree to which the anion can approach the IHP is unlikely to change[50].

5.4.2.2 Influences on Chaotropic Anions

Chaotropic anions (iodide, thiocyanate and perchlorate) have little influence on the structure of the water surrounding them. Thus any structure to the water surrounding the chaotropic anions is likely to be dominated by the bulk solution water. The retention of the chaotropic anions increased with IPA concentration (Sec. 5.4.1.2). This suggests an increased interaction between the anions and the double layer. In order to increase the interactions the anion must penetrate further into the double layer. Based on the work of James and Healy[50] there must be a decrease in the energy required to remove the surrounding water in order to favour greater penetration.

This increased penetration is a consequence of an increased disruption in the hydrogen bonding of the three layers of water surrounding the anion. In the instance of chaotropic anions the only significant source of water structure surrounding the anion comes from layer L_3 and the bulk solution. This structure subsequently influences the water of L_2, as the L_1 water structure is disrupted by the chaotropic anion. The presence of an IPA in the L_3 region would lead to a disruption of the hydrogen bonding structure in that region and consequently in the L_2 region. The net result is a significant reduction in the structure of water in all layers surrounding the chaotropic anions. This reduces the energy required to strip the water from the chaotropic anions, thus increasing the ability of the chaotropic anions to interact with the IPA. In addition the difference in the chaotropic nature of the IPAs is once again evident through the slopes of the retention factors (Fig. 5.5-5.7). The most chaotropic IPA has the greatest impact on the retention as the concentrations are changed.

5.4.2.3 Influences on Intermediate Monovalent Anions

Like the chaotropic anions the intermediate anions (bromide and nitrate) also experienced an increase in retention factor as the concentration of the IPA increases (Sec. 5.4.1.3). As the intermediate monovalent anions follow the same pattern as was seen for the chaotropic anions it is logical to presume the same mechanism is involved. Clearly the intermediate anions are not influenced to the same degree as the chaotropic anions. This reveals that though an anion may have chaotropic characteristics, the degree of the chaotropic character (i.e., position in the Hofmeister series) will influence its behaviour in IPC.

The mechanism for the interaction of the IPA and the intermediate monovalent anions is identical to that of the chaotropic anions. As the intermediate anions have less chaotropic character than the chaotropic anions the structure of water in the L_1 region is greater. Consequently the influence of the IPA is reduced. The chaotropic nature of the IPAs can be distinguished from the resulting impact on the retention factor of the intermediate anions. The most chaotropic of the IPAs, TBP, exhibited the greatest increase in retention factor for the two intermediate anions (Sec. 5.4.1.3).

It can be inferred from the data collected with the chaotropic anions that the influence of TBS on the intermediate anions would be similar to that of TBA and TBP. As it was seen with the chaotropic anions (Sec. 5.4.2.2) TBS has similar effects on chaotropic anions, though the magnitude of the effect is attenuated due to the more kosmotropic nature of TBS.

5.4.2.4 Influences on the Multivalent Anions

It was shown in Section 5.4.1.4 that the retention of the multivalent anions was unique. Whereas all other anion classes experienced an increase in retention factor irregardless of the IPA, the multivalent kosmotropic anions experienced differing behaviours dependant on the IPA. An understanding of the influences that govern this change in retention factor requires a further look at the model of anion solvation proposed by Collins and Washabaugh[23], as well as that of James and Healy[50].

It is important to note that a decrease in the retention factor of sulphate has previously been observed by Washabaugh and Collins[28]. In their experiment the retention factor of sulphate on a Sephadex G-10® size exclusion column was observed. The eluent conditions were varied in order to observe the retention factor changes with different amounts of one of three anions (sulphate, chloride and thiocyanate). The eluent was varied from 0 to 0.60 M concentrations of the anions. In order to differentiate between the analyte and eluent sulphate, isotopic ^{35}S was used for the analyte sulphate. With the addition of sulphate to the eluent the retention factors of ^{35}S sulphate increased. The addition of chloride to the eluent did not alter the retention factor of ^{35}S sulphate. When thiocyanate was added to the eluent the retention factor of ^{35}S sulphate was decreased. These alterations in retention were ascribed to the influence of the eluent ion on the water surrounding the analyte anion. In particular Washabaugh and Collins claimed that the addition of thiocyanate to the eluent enhances the hydrodynamic radius of sulphate anions, thus causing the lower retention factor on the size exclusion column[28].

The enhancement in hydrodynamic radius can be explained through the influence of an ion in the bulk phase on the water surrounding an anion of interest, as we have proposed in Section 5.4.2.1. It is clear through the Hofmeister series that thiocyanate is a chaotropic anion unlike chloride and sulphate which are clearly kosmotropic[22, 26, 28, 30]. It has also been established that the L_3 region of water molecules can contain salts[22, 23]. Through the combination of these two factors thiocyanate is capable of changing the hydrated radius of the sulphate anion, as seen by Washabaugh and Collins[28]. The influence of the analyte anion on the hydrogen bonding structure of L_1 and L_2 will depend on the chaotropic nature of the ion in L_3.

The work of James and Healy[48-50] clearly illustrates that the extent of ion-solvent interactions will dictate the ability of the ion to penetrate a double-layer. As I have previously established in Sections 5.4.2.1-3 this can be related to the differences seen in the retention of different anions from the Hofmeister series. It has been established that both TBP and TBA are chaotropic, with TBP being the most chaotropic of the two IPAs. With this consideration the decrease in retention factor for the multivalent anions (Sec. 5.4.1.4) can be compared to the results seen with thiocyanate[28]. Though the IPAs are cationic their chaotropic character will still influence the hydrogen bonding around the analyte anion. This will result in an increase in the ion-solvent interactions for the multivalent analytes, decreasing their retention factors.

As compared to the more chaotropic IPAs, TBS affected the retention of the multivalent anions in a significantly different manner. With TBS sulphate and thiosulphate were seen to, undergo drastically different changes in retention when the concentration of TBS is increased. For sulphate there was very little change in the retention with TBS, much as was seen with the monovalent kosmotropic anions. Lack of change in the retention can be attributed to the kosmotropic nature of sulphate. As such there is a strong hydrogen bonding network present through the L$_1$ and L$_2$ water layers around sulphate. To alter this structure a highly chaotropic ion in L$_3$ could disrupt some of the structure in L$_2$, which is what occurred with TBA and TBP. TBS however is less chaotropic; as a consequence it is unable to disrupt the water structure in L$_2$. Thus the retention of sulphate remains constant, independent of the TBS concentration.

The position of thiosulphate within the Hofmeister series has not been reported. However, it can be inferred from its IPC behaviour. Thiosulphate increases in retention with increases in the concentration of TBS. This indicates a significantly less kosmotropic character than sulphate. The hydrogen bonding structure around thiosulphate in a purely aqueous environment is dependant upon the competition between L$_1$ and L$_3$ for the bonding structure of L$_2$. When TBS is present in the eluent the mild chaotropic character of the IPA leads to a decrease in the hydrogen bonding structure in the bulk solution. The presence of the IPA in the L_3 region disrupts the hydrogen bonding structure in that region as well as disrupting the bonding structure in the L_2 region. The net effect is a decrease in the hydrogen bonding structure of the water around the thiosulphate anion, resulting in greater penetration of the thiosulphate into the double layer. This in turn leads to an increase in retention of the anion as the TBS concentration is increased.

5.4.2.1 Influence of IPA Hofmeister Character on Retention

It can be seen that the retention of the all anions is dependant on the relative kosmotropic/chaotropic characteristics of both the anion and the IPA. Thiosulphate acts as a particularly effective example of the differences that can be achieved through different combinations kosmotropic/chaotropic characteristics. When an IPA of sufficiently kosmotropic qualities is used the retention of thiosulphate will increase as the concentration of the IPA is increased. However if the IPA is sufficiently chaotropic the retention factor of thiosulphate will decrease with increasing IPA concentration.

This offers the potential that should the data be available to compare the kosmotropic/chaotropic characteristics (i.e. entropy values in water) of both the analyte and IPA, one could predict the impact upon retention due to changes in concentration of the IPA. The potential exists that such an equation would reveal an inversion value, at which point the ratio of characteristics of the anion and IPA reach a critical value that inverts the trend, as was seen with thiosulphate and TBS. This would allow for much more selective control of the retention factor of a desired anion.

5.4.3 Relative Retention

In the discussion above it has been shown that the different IPAs provide different retention for the anions studied. However it is not clear whether there are any selectivity differences offered by the three IPAs. The relative retention (r) can be used to quantify changes in selectivity. The relative retention is the ratio of the retention factor (k') of two analytes:

$$r = k_i'/k_R' \tag{5.1}$$

where k_i' is the retention factor for analyte i and k_R' is the retention factor for the reference ion (*i.e.*, chloride). By using the relative retention values, we are able to factor out the retention due to non-specific interactions, since they should be proportional for each analyte.

5.4.3.1 Influence of IPA on Relative Retention

As we have already examined, the retention factors of the anions changed with the increase in concentration of the IPA. The relative retention will aid in establishing if this change was in fact due to a change in selectivity or an increase in capacity of the column. Plots of relative retentions versus the IPA concentration for the chaotropic, intermediate and multivalent anions are shown in Figures 5.13, 5.14 and 5.15. The monovalent kosmotropic anions are not plotted as they all experienced near identical changes in retention factors. Thus no significant selectivity changes were achieved by changing the type of IPA.

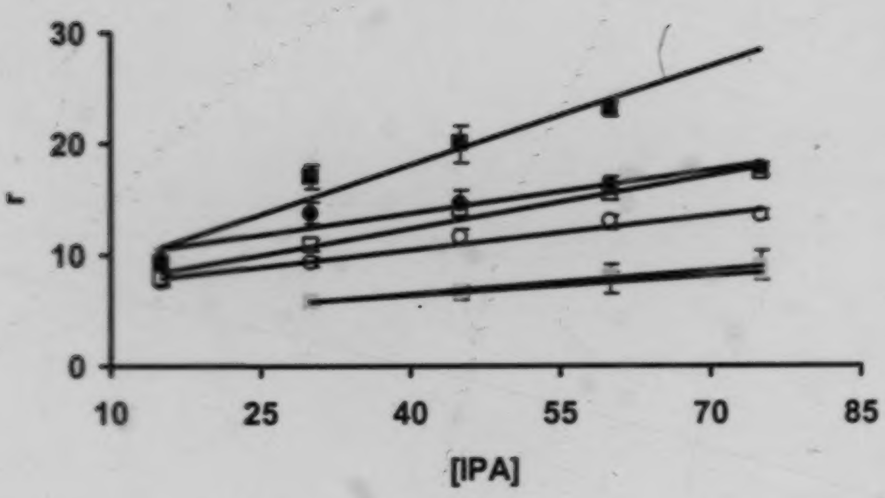


Figure 5.13 Relative retention of perchlorate (■) and thiocyanate (●) versus chloride for TBA (white), TBP (black) and TBS (grey). Error bars represent one standard deviation. **Elution conditions:** 30% methanol, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 µm), flow 0.3 ml/min

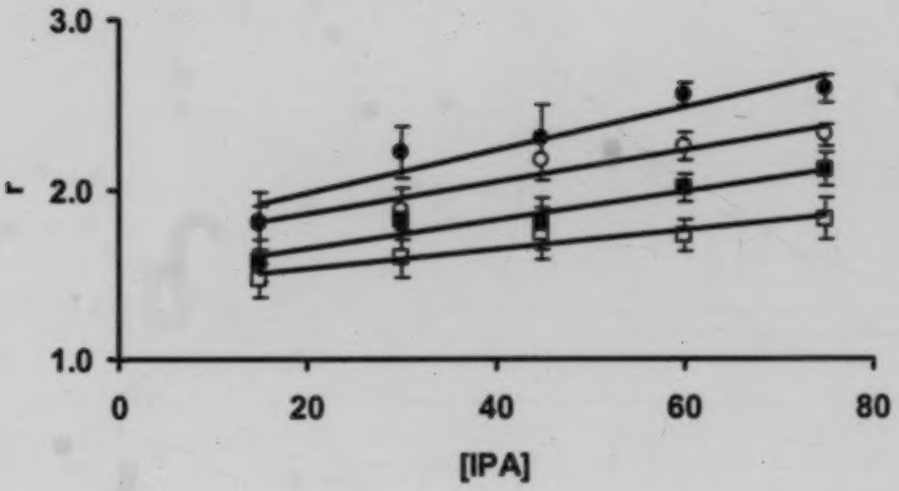


Figure 5.14 Relative retention of bromide (■) and nitrate (●) versus chloride for TBA (white) and TBP (black). Error bars represent one standard deviation. **Elution conditions:** 30% methanol, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 µm), flow 0.3 ml/min

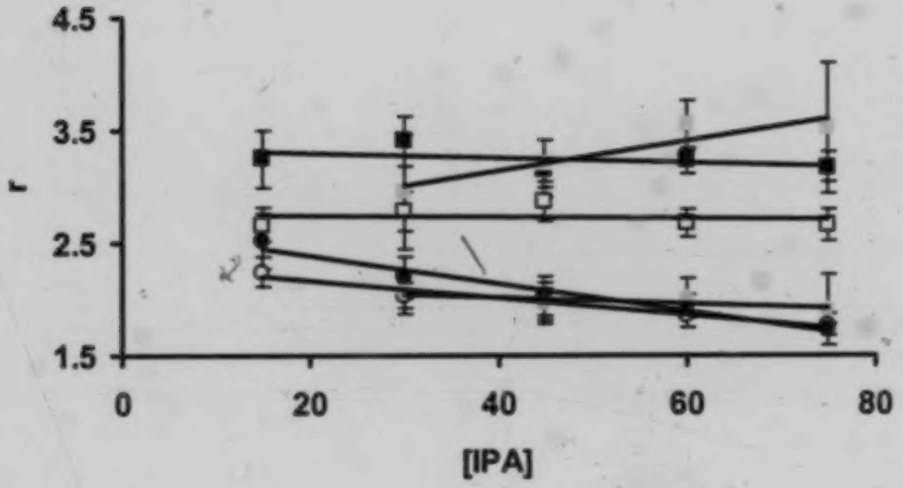


Figure 5.15 Relative retention of thiosulphate (■) and sulphate (●) versus chloride for TBA (white), TBP (black) and TBS (grey). Error bars represent one standard deviation. **Elution conditions:** 30% methanol, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 μm), flow 0.3 ml/min

5.4.3.2 Relative Retention of Chaotropic Anions

Figure 5.13 plots the relative retention for a selection of the chaotropic anions with the three IPAs. Iodide was omitted for clarity, but showed similar behaviour. It is clear from Figure 5.13 that TBP has a greater relative retention for the chaotropic anions than either of the other IPAs.

Figure 5.13 also shows that the relative retention increases with increasing IPA concentration regardless of the type of IPA. The degree to which relative retention increases correlates with the chaotropic character of the IPA (i.e., TBS ≤ TBA < TBP). However we can also see that the different IPAs provide different selectivity between chaotropic anions. For TBS there is nearly no selectivity difference between perchlorate and thiocyanate. Conversely TBP offers the greatest difference in relative retention between perchlorate and thiocyanate, with their relative retentions diverging further as the concentration of TBP is increased. TBA exhibits comparable influences to those seen for TBP, yet the magnitude of both the relative retention and their difference is not as great.

The slopes for the relative retention versus the concentration of TBS, TBA and TBP are: 0.07, 0.16 and 0.25 for perchlorate, 0.05, 0.11 and 0.12 for thiocyanate and 0.02, 0.04 and 0.06 for iodide respectively. It is clear from this that the greatest selectivity difference comes from the use of the most chaotropic IPA (TBP), and that further increases in this selectivity can be achieved through increases in the concentration of the IPA.

5.4.3.3 Relative Retention of Intermediate Anions

For the intermediate anions the relative retentions for both nitrate and bromide are plotted in Figure 5.14 for both TBA and TBP. As would be anticipated from the results of the chaotropic anions (Sec. 5.4.3.2), the relative retention increases as the IPA concentration is increased. TBP exhibits a greater relative retention for the intermediate anions relative to chloride.

Unlike the chaotropic anions, changing the IPA concentration has a minimal effect on the selectivity between nitrate and bromide. The slopes for bromide and nitrate were found to be 0.008 and 0.012 for TBP and 0.006 and 0.010 for TBA respectively. This suggests that as the chaotropic character of an anion decreases there will be a smaller less selectivity influence achieved through the use of more chaotropic IPAs.

5.4.3.4 Relative Retention of Multivalent Anions

The relative retention for the multivalent anions sulphate and thiosulphate are plotted in Figure 5.15, for all three IPAs. Chromate shows similar behaviour but was excluded for clarity. As was seen with the chaotropic and intermediate anions the relative retention of the multivalent anions is generally greatest with TBP. There is however a significant difference from the previous classes of anions, as the multivalent anions behave very differently with TBS (Sec. 5.4.2.4). In fact all possible scenarios are seen, with the relative retention factors increasing, decreasing and remaining constant, depending on the IPA-anion combination.

The most chaotropic IPA, TBP, causes a decrease in relative retention as the IPA concentration is increased for all the multivalent anions. The plot of the decrease in relative retention has slopes of -0.002, -0.004 and -0.014 for thiosulphate, chromate and sulphate respectively. For TBA, there is a smaller change in relative retention as the TBA concentration is increased, with both thiosulphate and chromate having minor decreases in retention factor. Sulphate however does decrease more significantly, with a slope of -0.006. But that is a much smaller slope than seen for sulphate with TBP (-0.014). The influence of the TBS concentration on the multivalent anions is the most interesting of the IPAs studied. TBS demonstrates a clear change in selectivity for the multivalent anions, both with respect monovalent kosmotropic anions and other multivalent anions. The increase in TBS concentration leads to a clear increase in the relative retention of thiosulphate while the relative retention for sulphate decreases. The respective slopes from the plot for those two multivalent anions are 0.013 and -0.003. Once again the slope for the decrease of sulphate is reduced with an IPA of more kosmotropic character.

5.4.3.5 Overall Impact on Relative Retention

The differences in relative retentions that are seen with the three IPAs illustrate that different separation patterns can be achieved by changing the IPA. For the most part the elution order within a class of anions remains the same, regardless of the IPA used. However, the relative retention between two anions in the same class may be adjusted, as most anions experienced the influence of the

IPAs with differing magnitudes (slope of the relative retention versus IPA concentration).

As the different classes of anions are influenced differently by the IPA the potential exists for some changes in elution order. For example the combination of the increasing retention factors of the intermediate anions and the decreasing retention factors of the multivalent species. The prominent decrease of sulphate leads it to elute before nitrate and bromide under the conditions examined for TBA and TBP. Further increases of the concentration in TBA and TBP lead to a cross-over between more intermediate and multivalent anions. The kosmotropic anions are virtually unchanged with all three IPAs, showing only slight changes in relative retention as the IPA concentration is changed. The chaotropic anions are affected to the greatest extent, with an increase in the relative retention factors as the concentration of the IPA is increased. Yet through this increase there is improved separation between chaotropic anions it comes however at the expense of longer retention times.

5.5 Effect of Methanol Percentage on Anion Retention

5.5.1 Effect of Methanol on Monovalent Kosmotropic Anions

Increasing methanol with all three IPAs resulted in a decrease in the retention of all of the monovalent kosmotropic anions (iodate, chloride and nitrite). The results are tabulated in Tables 5.4-5.6 and presented graphically as the log k' versus the percent methanol in Figures 5.16-5.18. A plot of the log k' versus the percent methanol is anticipated to yield a linear relationship for a hydrophobic analyte on a reversed phase column.

Table 5.4 Retention factors for various percentages of methanol with constant tetrabutylammonium bicarbonate[a]

	Retention Factor (One Standard Deviation)			
	10%	**20%**	**30%**	**40%**
Iodate	0.192 (0.002)	0.140 (0.010)	0.150 (0.006)	0.127 (0.022)
Chloride	0.357 (0.015)	0.205 (0.008)	0.210 (0.009)	0.234 (0.010)
Bromide	0.754 (0.005)	0.388 (0.009)	0.218 (0.002)	0.242 (0.006)
Nitrite	0.629 (0.025)	0.332 (0.013)	0.307 (0.017)	0.199 (0.014)
Nitrate	0.892 (0.010)	0.409 (0.027)	0.378 (0.012)	0.225 (0.010)
Sulfate	0.927 (0.018)	0.413 (0.018)	0.471 (0.019)	0.209 (0.010)
Chromate	1.258 (0.043)	0.472 (0.010)	0.529 (0.016)	0.226 (0.012)
Thiosulfate	1.288 (0.019)	0.492 (0.019)	0.559 (0.019)	0.223 (0.010)
Iodide	2.240 (0.064)	0.891 (0.027)	0.713 (0.024)	0.304 (0.017)
Thiocyanate	7.497 (0.214)	2.328 (0.044)	1.592 (0.006)	0.457 (0.012)
Perchlorate	7.393 (0.207)	2.658 (0.058)	1.645 (0.027)	0.489 (0.041)

a. **Elution conditions:** 15 mM TBA, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 μm), flow 0.3 ml/min

Table 5.5 Retention factors for various percentages of methanol with constant tetrabutylphosphonium bicarbonate[a]

	Retention Factor (One Standard Deviation)			
	10%	**20%**	**30%**	**40%**
Iodate	0.308 (0.004)	0.246 (0.003)	0.185 (0.005)	0.138 (0.003)
Chloride	0.712 (0.008)	0.481 (0.006)	0.325 (0.017)	0.200 (0.011)
Bromide	1.437 (0.013)	0.861 (0.010)	0.530 (0.009)	0.315 (0.011)
Nitrate	2.190 (0.023)	1.129 (0.010)	0.620 (0.019)	0.0359 (0.014)
Sulfate	2.454 (0.021)	1.392 (0.014)	0.753 (0.017)	0.396 (0.011)
Chromate	3.604 (0.063)	1.898 (0.027)	1.013 (0.022)	0.499 (0.020)
Thiosulfate	4.264 (0.067)	2.177 (0.026)	1.096 (0.011)	0.535 (0.008)
Iodide	8.229 (0.072)	3.411 (0.033)	1.488 (0.018)	0.703 (0.009)
Thiocyanate	-	9.075 (0.073)	3.130 (0.038)	1.202 (0.017)
Perchlorate	-	11.006 (0.053)	3.771 (0.067)	1.426 (0.025)

a. **Elution conditions:** 15 mM TBP, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 µm), flow 0.3 ml/min

Table 5.6 Retention factors for various percentages of methanol with constant tributylsulphonium bicarbonate[a]

Retention Factor (One Standard Deviation)		
	10%	**20%**
Iodate	0.182 (0.007)	0.113 (0.004)
Chloride	0.243 (0.003)	0.151 (0.003)
Bromide	0.243 (0.005)	0.317 (0.004)
Nitrate	0.303 (0.005)	0.155 (0.002)
Sulfate	0.341 (0.003)	0.184 (0.008)
Chromate	0.532 (0.004)	0.208 (0.004)
Thiosulfate	0.941 (0.005)	0.251 (0.003)
Iodide	0.979 (0.009)	0.256 (0.003)
Thiocyanate	1.028 (0.037)	0.311 (0.004)
Perchlorate	2.235 (0.017)	0.849 (0.011)

a. **Elution conditions:** 15 mM TBS, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 μm), flow 0.3 ml/min

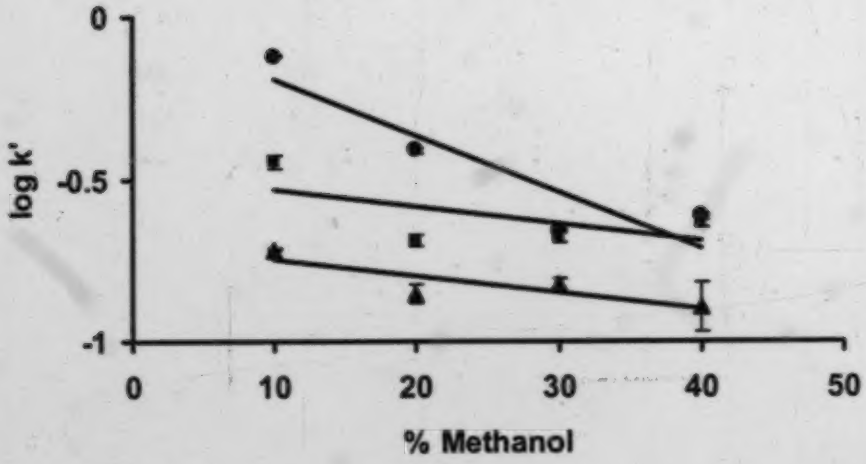


Figure 5.16 Log of the retention factors of iodate (▲), chloride (■) and nitrite (●) versus the percent methanol in the eluent. Error bars represent one standard deviation. **Elution conditions:** 15 mM TBA, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 µm), flow 0.3 ml/min

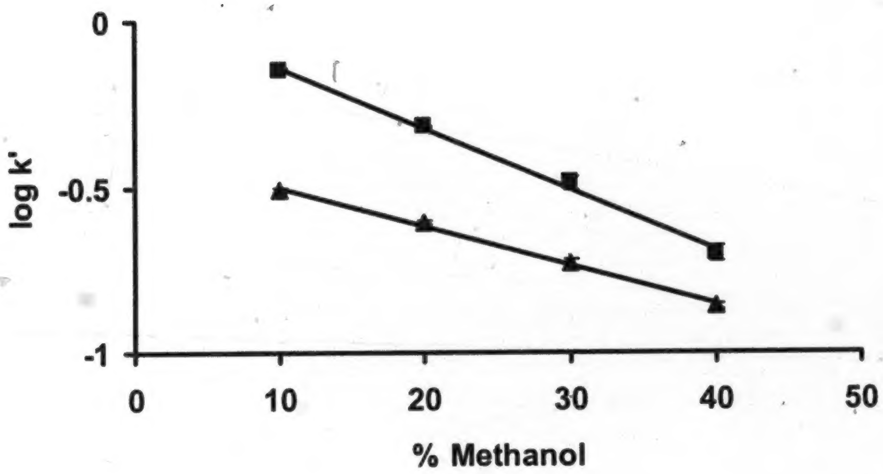


Figure 5.17 Log of the retention factors of iodate (▲) and chloride (■) versus the percent methanol in the eluent. Error bars represent one standard deviation. **Elution conditions:** 15 mM TBP, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 µm), flow 0.3 ml/min

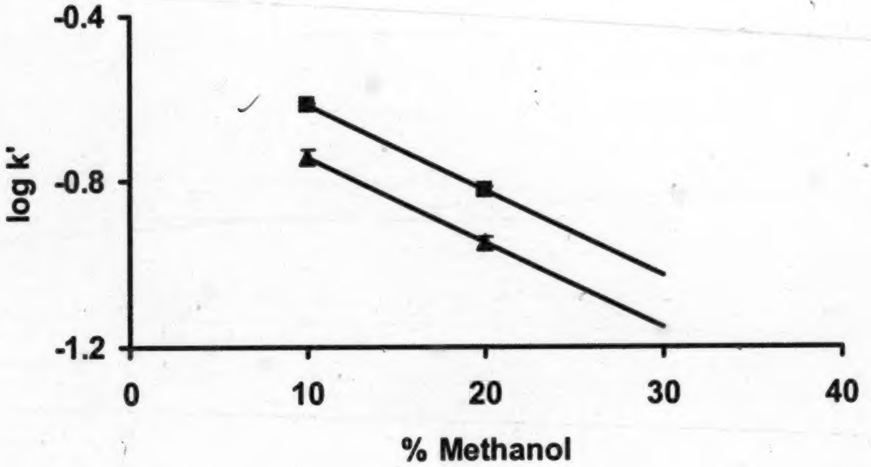


Figure 5.18 Log of the retention factors of iodate (▲), chloride (■) and nitrite (●) versus the percent methanol in the eluent. Error bars represent one standard deviation. **Elution conditions:** 15 mM TBS, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 μm), flow 0.3 ml/min

In Figures 5.16-5.18 it is evident that the overall impact of increasing the percentage of methanol is a decrease in the retention of monovalent kosmotropic anions. With each IPA there are some differences in the scale of the impact of increasing the amount of methanol in the eluent. With TBP the decrease in retention factor for iodate and chloride (nitrite was not studied), with slopes of -0.012 and -0.018 respectively. For TBA the anions deviate significantly from the expected linear trend. Both chloride and nitrite exhibit relative increases in retention at the higher methanol percentages. Overall however increasing methanol decreases the retention factor. The slopes from a linear trend line for iodate, chloride and nitrate with TBA are -0.005, -0.006 and -0.017 respectively.

For TBS data could only be collected below 20% methanol due to the lower retention observed with this IPA. Regardless, retention decreases with increasing methanol (Figure 5.16). However nitrite appears to increase in retention at 20% methanol, similar to what was seen at 40% methanol with TBA.

When the slopes in Figures 5.16 and 5.17 for TBA and TBP (TBS is excluded due to the limited data) are compared, it is evident that there is a significant difference between the two IPAs. For TBP, the rate of decrease is almost 3 times greater for iodate and chloride than with TBA.

5.5.2 Effect of Methanol on Monovalent Chaotropic Anions

The effect of methanol on the retention of monovalent chaotropic anions is summarized in Tables 5.4-5.6 and Figures 5.19-5.21. Similarly to the kosmotropic anions, the chaotropic anions (iodide, thiocyanate and perchlorate) decrease in retention as the percent methanol is increased. Unlike the

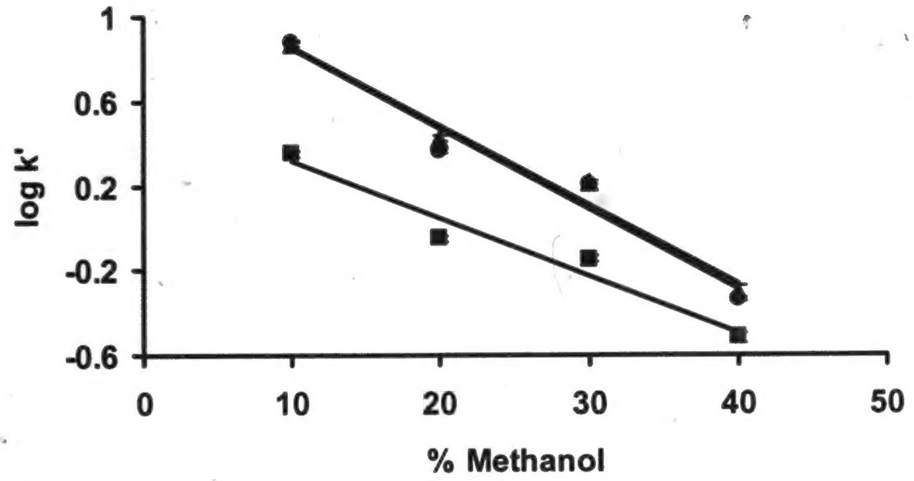


Figure 5.19 Log of the retention factors of iodide (■), thiocyanate (●) and perchlorate (▲) versus the percent methanol in the eluent. Error bars represent one standard deviation. **Elution conditions:** 15 mM TBA, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 µm), flow 0.3 ml/min

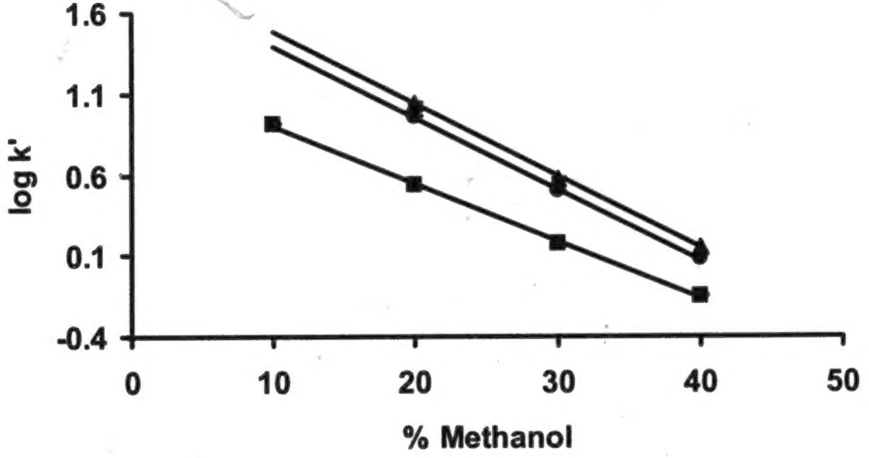


Figure 5.12 Log of the retention factors of iodide (■), thiocyanate (●) and perchlorate (▲) versus the percent methanol in the eluent. Error bars represent one standard deviation. **Elution conditions:** 15 mM TBP, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 μm), flow 0.3 ml/min

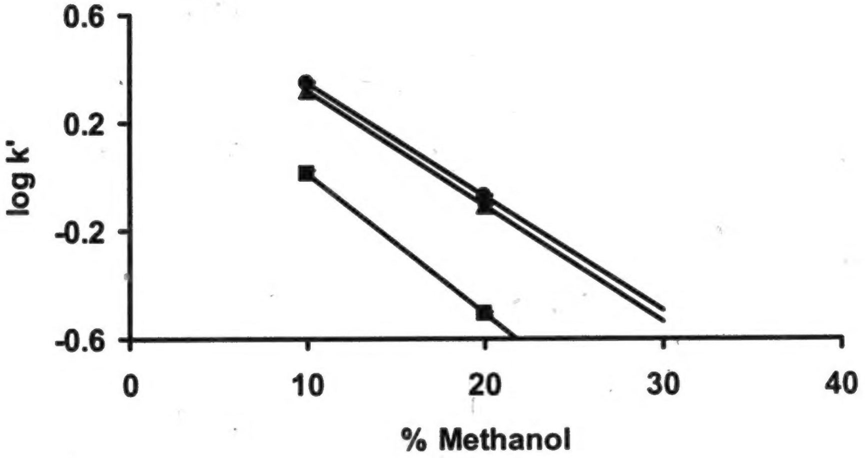


Figure 5.21 Log of the retention factors of iodide (■), thiocyanate (●) and perchlorate (▲) versus the percent methanol in the eluent. Error bars represent one standard deviation. **Elution conditions:** 15 mM TBS, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 µm), flow 0.3 ml/min

kosmotropic anions the chaotropic anions uniformly decrease in retention for all the IPAs. The slope of the plot for the retention of perchlorate and thiocyanate were almost identical both for TBA and TBP. With TBA the slopes were -0.038 and -0.038 respectively. With TBP the slopes for perchlorate and thiocyanate were identical at -0.044. Additionally the slope for iodide was always the least of the three anions regardless of the IPA. As anticipated the k' with TBP were the greatest and TBS yielded the least retention of the chaotropic anions.

5.5.3 Effect of Methanol on Intermediate Monovalent Anions

Retention data for the intermediate monovalent anions is tabulated in Tables 5.4-5.6 and plotted in Figures 5.22-5.25. Both nitrate and bromide undergo significant decreases in retention with increasing methanol with all of the IPAs. As with the chaotropic anions, the rate of decrease in retention is dependant upon the IPA, with TBP again providing the greatest change over the range studied. However the slopes for the decrease in retention factor differ between the two intermediate anions, with nitrate typically experiencing the greatest rate of decrease. For TBA the slope for bromide was -0.015 compared to -0.019 for nitrate. Similarly with TBP the slopes were seen to be -0.022 for bromide and -0.026 for nitrate. Thus changes in the percent methanol allows for some minor change in selectivity.

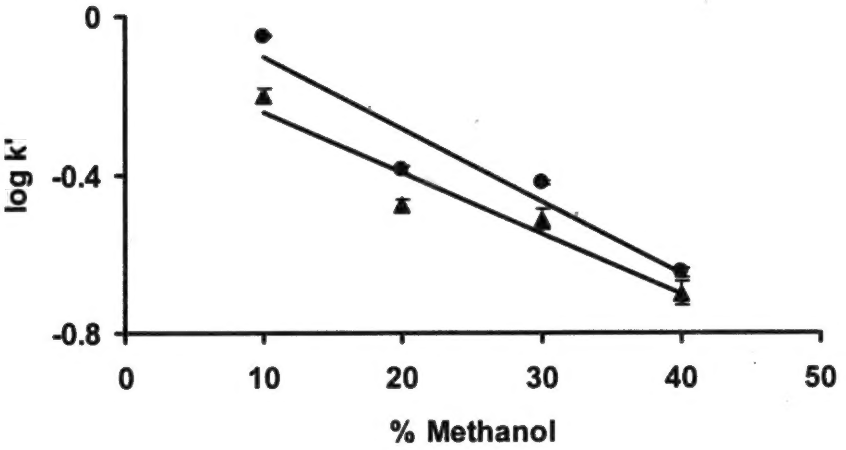


Figure 5.22 Log of the retention factors of nitrate (●) and bromide (▲) versus the percent methanol in the eluent. Error bars represent one standard deviation. **Elution conditions:** 15 mM TBA, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 μm), flow 0.3 ml/min

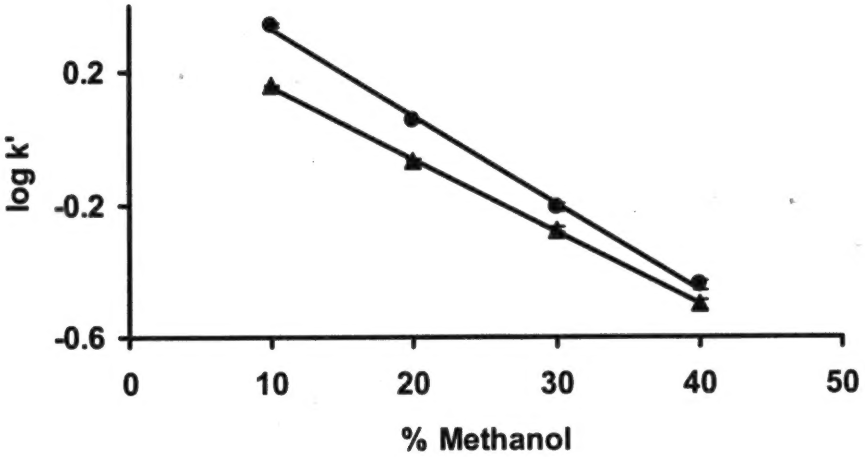


Figure 5.23 Log of the retention factors of nitrate (●) and bromide (▲) versus the percent methanol in the eluent. Error bars represent one standard deviation. **Elution conditions:** 15 mM TBP, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 μm), flow 0.3 ml/min

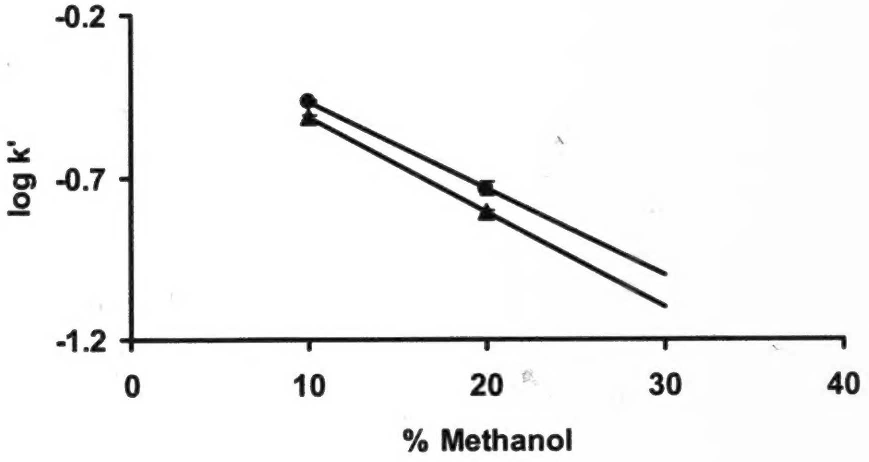


Figure 5.23 Log of the retention factors of nitrate (●) and bromide (▲) versus the percent methanol in the eluent. Error bars represent one standard deviation. **Elution conditions:** 15 mM TBS, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 µm), flow 0.3 ml/min

5.5.4 Effect of Methanol on Multivalent Anions

Retention data for sulphate, thiosulphate and chromate are tabulated in Tables 5.4-5.6 and plotted in Figures 5.24-5.27. Once again there is a clear decrease in retention with percent methanol for all the IPAs. However there are also dramatic selectivity changes within the doubly-charged anions. Interestingly this is only seen with TBA and TBS. There is no evidence of a selectivity change with TBP. For TBP, the log k' of all three anions experience similar rates of decrease over the range studied. Thiosulphate has a slope of -0.030, chromate a slope of -0.029 and sulphate a slope of -0.027 with TBP. With TBA the rate of change in log k' versus the methanol percentage is greatest for thiosulphate and chromate, both having nearly identical values, with slopes of -0.023. The slope for sulphate however is almost half that value at -0.014. As a result, sulphate is the first of the multivalent anions eluted at 10% methanol, while sulphate elutes after chromate and thiosulphate at 40% methanol. This selectivity is similar to that seen with TBS in 20% methanol.

5.5.5 Overall Effect of Methanol on Ion Pair Chromatography

Addition of methanol to the eluent reduces the retention times of all inorganic anions in IPC. This effect is universal to all the IPAs studied. However, the more chaotropic the IPA, the greater the retention at low methanol and the greater the resultant decrease in log k' with increasing methanol. As a consequence, little to no control of the selectivity can be achieved by altering the methanol concentration. Changing the percent methanol is essentially just a means of controlling the overall retention times.

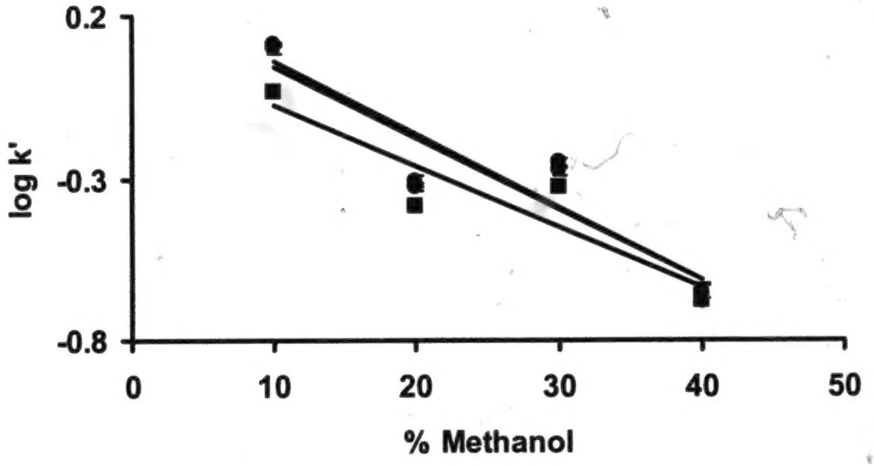


Figure 5.25 Log of the retention factors of sulphate (■), chromate (●) and thiosulphate (▲) versus the percent methanol in the eluent. Error bars represent one standard deviation. **Elution conditions:** 15 mM TBA, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 μm), flow 0.3 ml/min

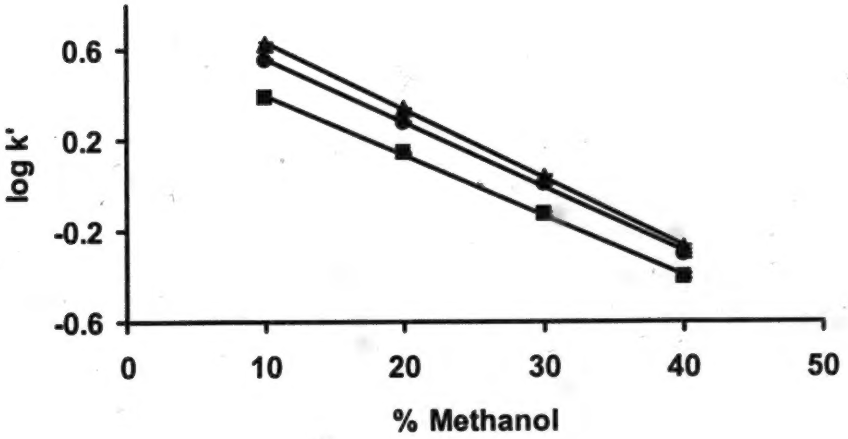


Figure 5.26 Log of the retention factors of sulphate (■), chromate (●) and thiosulphate (▲) versus the percent methanol in the eluent. Error bars represent one standard deviation. **Elution conditions:** 15 mM TBP, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 μm), flow 0.3 ml/min

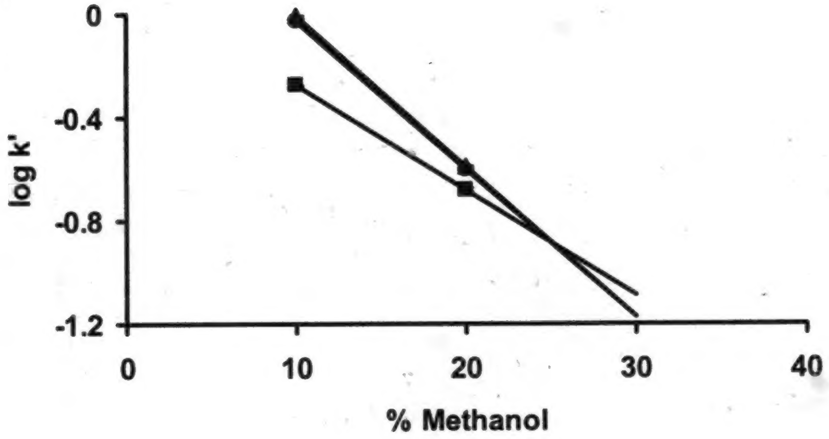


Figure 5.27 Log of the retention factors of sulphate (■), chromate (●) and thiosulphate (▲) versus the percent methanol in the eluent. Error bars represent one standard deviation. **Elution conditions:** 15 mM TBS, 20 mM $NaHCO_3$, 30 °C, pH 7.5, Xterra MS C_{18} (2.1 x 100 mm, 3.5 μm), flow 0.3 ml/min

One exception does produce a selectivity change. At appropriate methanol percentages sulphate was more retained than both thiosulphate and chromate, which under most circumstances elute after sulphate. This was only achieved with TBA and TBS at 40% and 20% methanol respectively. The change in selectivity is surprising as only one anion experiences this change out of all those analyzed.

A hypothesis for this change relates to the kosmotropic/chaotropic character of both the anion and the IPA. Sulphate is the most kosmotropic of the multivalent anions (Section 5.4.1.4) and is one of the most kosmotropic anions in the Hofmeister scale (eqn 5.1)[23]. TBA has been established as being less chaotropic than TBP and TBS is the least chaotropic of the IPA (Sec. 5.2.1.2). Hence, the combination of kosmotropic analytes and kosmotropic IPAs are required for this selectivity change. The change in selectivity occurred gradually as the percentage of methanol in the eluent was changed. In Sections 5.5.2.2.1-5.5.2.2.4 the presence of methanol was shown to have a number of effects on the double-layer mechanism for ion pair retention. It is important to note that the selectivity change required different amounts of methanol for the different IPAs. It appears to be the combination of these influences and the character of the analyte and IPA that leads to a selectivity change.

5.6 Influence of Methanol on IPA Separations

5.6.1 Influence of Methanol on the IPA Equilibrium

As in all ion-pair chromatographic systems the eluent contains an organic solvent to control the adsorption of the IPA to the stationary phase[47, 52, 53]. In my study methanol was employed. The mechanism of retention of the IPA to the column is the same as in reversed phase chromatography (Section 1.3.2). The amount of methanol alters the concentration of the IPA adsorbed. Bartha and Vigh studied the effect of methanol on the adsorption of a variety of ammonium IPAs possessing different alkyl chains[52]. They concluded that the longer chained ammonium salts (tetrabutyl and tetrapropyl) provided higher surface concentrations than tetraethyl and tetramethyl at the same eluent concentration and methanol percentage. In addition they found that increases in the percentage of methanol in the eluent decreased the concentration of the IPA in the stationary phase.

In my experiments the effect of methanol was studied between 10 and 40% v/v. Higher values were not explored as retention becomes so low that no separation can be achieved. This is consistent with Bartha and Vigh[52] who observed that in 50% MeOH the amount of adsorbed IPA was less than 15% of the amount adsorbed in the absence of methanol. For my experiment constant concentrations of IPA (15 mM) and counter ion (20 mM) were used, the pH was 7.5 and the temperature was 30.0°C.

In reverse phase chromatography plots of log k' versus percent methanol are commonly linear[54]. Since anion retention in IPC is dependant on the adsorption of the IPA a similar plot is used to study the anion retention. With ion pair chromatography, where reversed phase columns are used, similar linear plots have been observed[51, 53]. However Bartha and Vigh[53] did observe instances where there was some deviation from linearity, particularly with analytes that were less hydrophobic.

Theories regarding the influence of organic modifiers on retention are primarily based on reversed phase chromatography[51, 55-58]. These theories focus on the adsorption of IPA onto the stationary phase. As the amount of adsorbed IPA decreases the retention of the ionic analytes also decreases. Intuitively this would be anticipated; as the mobile phase becomes less polar there is less retention of the IPA at the stationary phase. Consequently this impacts the double-layer model by reducing the amount of charged IPA at the stationary phase, reducing the potential at the surface and decreasing the strength of the double-layer.

In the work of Bartha *et al.*[56, 58] and Zou *et al.*[51] examined the influence of organic modifiers on the retention of anions through IPC. In all instances decreased retention was seen with increasing amounts of organic modifier, comparable to my work. And as was seen in my work, Figures 5.16 - 5.28, it is clear that the slope for log k' vs. percent organic modifier varies between the anion classes.

5.6.2 Influence on Water Structure

The influence of methanol on the equilibrium of the mobile phase and stationary phase IPAs sufficiently explains the overall decrease in retention factors observed. However the gradual increase in retention of nitrite and chloride with TBA, as well as the changes in selectivity for sulphate with TBA and TBS, are not accounted for by changes in the IPA equilibrium. As discussed in Section 5.4.2, the removal of the solvent from around the ion is key to its retention within the double-layer. I propose that the addition of an organic modifier to the eluent alters the solvation shells around the ions. Though likely a minor influence, as compared to the change in adsorption of the IPA, this may account for the deviations from the expected results.

The nature of water is such that the hydrogen bonding between individual water molecules is key to the overall structure of water[59]. As methanol is added to water there is a disruption of the orientation of water through alterations in the hydrogen bonding characteristics[60, 61]. Similar to water there is a polarity to methanol, leading to specific structural orientation in the liquid phase. Liquid water is considered to have a tetrahedral network of molecules[59-62] whereas the ordering of the molecules in liquid methanol is that of polymeric chains[60, 61]. Due to these differences, a mixture of the two liquids will not retain its original molecular orientations. The resulting structure of methanol-water mixtures has been investigated both through simulations[61, 62] and physical measurements[60]. These studies show that the composition of the mixture influences the structure. At low concentrations of water, the methanol molecules surround and direct the hydrogen bonding of the water molecules[60]. When the concentration of water is the greater than that of methanol, the presence of methanol enhances the localized structuring of water. Simulations found significant water structures forming around the hydroxyl region of methanol molecules[61]. In addition the study of Hawlicka *et al.*[62] investigated the solvation of sodium chloride in methanol-water solutions of 10 and 90 mol% methanol. Hawlicka *et al.* concluded that there was preferential solvation of the ions which was dependant upon the mixture composition. At 10 mol% methanol there was preferential solvation of both sodium and chloride by methanol. However when the methanol was increased to 90 mol% the preferential solvation of sodium was not seen whereas the preferential solvation of chloride by methanol continued[62].

As was shown with the changes in the concentration of the IPAs, Section 5.4.2, the structure of water around the anion is key to its retention. Additionally the potential exists for changes in the ion-solvent interactions as the methanol content is changed. Changes in the strength of the ion-solvent interactions, to varying degrees dependant upon the anion class (kosmotropic vs. chaotropic). The differences seen in the decreases in retention Figures 5.16, 5.26 and 5.28 could be the result of a decreased ion-solvent interaction, allowing for greater penetration into the double-layer, consequently increased retention.

5.7 Conclusions

This work has shown the capabilities of new IPAs, tetrabutylphosphonium and tributylsulphonium, for the separation of anions by ion pair chromatography. Significant changes in retention characteristics are observed with these new IPAs (Fig. 5.28). TBS provides less retention than traditional TBA, and TBP provides greater retention. In addition changes in the concentration of the IPA have different influences, dependant upon the anion. For TBP and TBA increases in

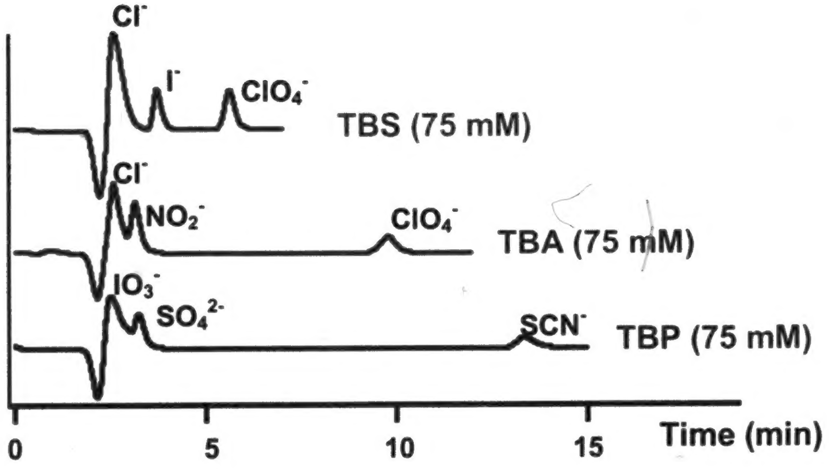


Figure 5.28 Separation of selections of kosmotropic and chaotropic anions through IPC with various IPAs. **Eluent Conditions:** 30% MeOH, 20 mM NaHCO3, pH 7.5, temp 30 °C, Xterra MS C_{18} (2.1x100mm, 3.5 μm), 0.2 ml/min

IPA concentration lead to an increase in retention of the more chaotropic anions. For the multivalent kosmotropic anions retention decreased more with increased TBP concentration than was seen with TBA. TBS was particularly interesting as it provided a glimpse of the influence of the Hofmeister series on ion pair separations. The unique effect of increasing the retention factor of thiosulphate while the concentration of TBS was increased was related to these Hofmeister effects. In addition methanol was also revealed to have a unique influence on the selectivity of sulphate when TBA or TBS was used as the IPA. The ability to cause a selectivity change among the multivalent kosmotropic anions was related to the influence of methanol, though establishment of a firm mechanism for this action is not feasible at this time. Nevertheless the Hofmeister characteristic of the IPA has been shown to be key in understanding and controlling the separation of all anions which span the Hofmeister series.

5.7 References

(1) Li, C. Y.; Gao, J. Z.; Yu, S. Y.; Han, X. Q.; Li, B. Y.; Liu, H. T. *Chromatographia* **2001**, *54*, 114-116.

(2) Karthikeyan, S.; Hirata, S. *Analytical Letters* **2003**, *36*, 2355-2366.

(3) Socher, G.; Nussbaum, R.; Rissler, K.; Lankmayr, E. *Chromatographia* **2001**, *54*, 65-70.

(4) Miwa, H. *Analytica Chimica Acta* **2002**, *465*, 237-255.

(5) Arnault, I.; Christides, J. P.; Mandon, N.; Haffner, T.; Kahane, R.; Auger, J. *Journal of Chromatography A* **2003**, *991*, 69-75.

(6) Ramm, M.; Wolfender, J. L.; Queiroz, E. F.; Hostettmann, K.; Hamburger, M. *Journal of Chromatography A* **2004**, *1034*, 139-148.

(7) Hearn, M. T. W. *Ion-Pair Chromatography, theory and biological and pharmaceutical applications*; Marcel Dekker, Inc.: New York, 1985.

(8) Dodds, E. D.; Kennish, J. M.; von Hippel, F. A.; Bernhardt, R.; Hines, M. E. *Analytical and Bioanalytical Chemistry* **2004**, *379*, 881-887.

(9) Hedrick, E.; Munch, D. *Journal of Chromatography A* **2004**, *1039*, 83-88.

(10) Liu, Y. J.; Mou, S. F. *Journal of Chromatography A* **2003**, *997*, 225-235.

(11) Tian, K.; Dasgupta, P. K.; Anderson, T. A. *Analytical Chemistry* **2003**, *75*, 701-706.

(12) Sharp, D. W. A. *The Penguin Dictionary of Chemistry*, Second ed.; Peguin Books: Toronto, 1990.

(13) Wrath, L. M.; Cooper, R. S.; Fritz, J. S. *Journal of Chromatography* **1989**, *479*, 401-409.

(14) Michigami, Y.; Fujii, K.; Ueda, K. *Journal of Chromatography A* **1994**, *644*, 117-122.

(15) Shapovalova, E. N.; Ofitserova, M. N.; Savot'yanova, E. V.; Shpigun, O. A. *Journal of Analytical Chemistry* **2001**, *56*, 181-187.

(16) Bartha, A.; Vigh, G.; Vargapuchony, Z. *Journal of Chromatography* **1990**, *499*, 423-434.

(17) Bartha, A.; Vigh, G. *Journal of Chromatography* **1987**, *395*, 503-509.

(18) Tavares, F. W.; Bratko, D.; Blanch, H. W.; Prausnitz, J. M. *Journal of Physical Chemistry B* **2004**, *108*, 9228-9235.

(19) Lizondo-Sabater, J.; Seguli, M. J.; Lloris, J. M.; Martinez-Manez, R.; Pardo, T.; Sancenon, F.; Soto, J. *Sensors and Actuators B-Chemical* **2004**, *101*, 20-27.

(20) Inoue, T.; Yokoyama, Y.; Zheng, L. Q. *Journal of Colloid and Interface Science* **2004**, *274*, 349-353.

(21) Bostrom, M.; Williams, D. R. M.; Ninham, B. W. *Biophysical Journal* **2003**, *85*, 686-694.

(22) Cacace, M. G.; Landau, E. M.; Ramsden, J. J. *Quarterly Reviews of Biophysics* **1997**, *30*, 241-277.

(23) Collins, K. D.; Washabaugh, M. W. *Quarterly Reviews of Biophysics* **1985**, *18*, 323-422.

(24) Cook, H. A.; Dicinoski, G. W.; Haddad, P. R. *Journal of Chromatography A* **2003**, *997*, 13-20.

(25) Roberts, J. M.; Diaz, A. R.; Fortin, D. T.; Friedle, J. M.; Piper, S. D. *Analytical Chemistry* **2002**, *74*, 4927-4932.

(26) Yokoyama, T.; Macka, M.; Haddad, P. R. *Analytica Chimica Acta* **2001**, *442*, 221-230.

(27) Cook, H. A.; Hu, W. Z.; Fritz, J. S.; Haddad, P. R. *Analytical Chemistry* **2001**, *73*, 3022-3027.

(28) Washabaugh, M. W.; Collins, K. D. *Journal of Biological Chemistry* **1986**, *261*, 2477-2485.

(29) Jiang, W.; Irgum, K. *Analytical Chemistry* **1999**, *71*, 333-344.

(30) Sun, Z. Y.; Yuan, R.; Chai, Y. Q.; Xu, L.; Gan, X. X.; Xu, W. J. *Analytical and Bioanalytical Chemistry* **2004**, *378*, 490-494.

(31) Haddad, P. R.; E, J. P. *Ion Chromatography: principals and applications*, 1st ed.; Elsevier: Amsterdam, 1990.

(32) Valenzuela, F. A.; Green, T. K.; Dahl, D. B. *Journal of Chromatography A* **1998**, *802*, 395-398.

(33) Hoffman, J. L. *Journal of Chromatography* **1991**, *588*, 211-216.

(34) Aced, G.; Mockel, H. J.; Nelsen, S. F. *Journal of Liquid Chromatography* **1989**, *12*, 3201-3218.

(35) Wandlowski, T.; Marecek, V.; Samec, Z.; Fuoco, R. *Journal of Electroanalytical Chemistry* **1992**, *331*, 765-782.

(36) Hirayama, N.; Umehara, W.; Makizawa, H.; Honjo, T. *Analytica Chimica Acta* **2000**, *409*, 17-26.

(37) Umemura, T.; Kamiya, S.; Itoh, A.; Chiba, K.; Haraguchi, H. *Analytica Chimica Acta* **1997**, *349*, 231-238.

(38) Hu, W. Z.; Yang, P. J.; Hasebe, K.; Haddad, P. R.; Tanaka, K. *Journal of Chromatography A* **2002**, *956*, 103-107.

(39) Hu, W. Z.; Hasebe, K.; Ding, M. Y.; Tanaka, K. *Fresenius Journal of Analytical Chemistry* **2001**, *371*, 1109-1112.

(40) Hu, W. Z.; Tanaka, K.; Hasebe, K. *Analyst* **2000**, *125*, 447-451.

(41) Lucy, C. A.; Hatsis, P. In *Chromatography 6th edition, fundamentals and applications of chromatography and related differential migration methods, part A: fundamentals and techniques*; Heftmann, E., Ed.; Elsevier: Amsterdam, 2004; Vol. 69A, pp 518.

(42) Madden, J. E.; Shaw, M. J.; Dicinoski, G. W.; Avdalovic, N.; Haddad, P. R. *Analytical Chemistry* **2002**, *74*, 6023-6030.

(43) Milligan, T. W.; Minor, B. C. *Journal of Organic Chemistry* **1963**, *28*, 235-236.

(44) Okada, T. *Journal of Chromatography A* **1997**, *780*, 343-360.

(45) Madambatan, L. S.; Strasters, J. K.; Khaledi, M. G. *Journal of Chromatography A* **1994**, *683*, 321-334.

(46) Fritz, J. S.; Gjerde, D. T. *Ion Chromatography*, 3rd ed.; Wieley-Vch: Weinheim, 2000.

(47) Chen, J. G.; Weber, S. G.; Glavina, L. L.; Cantwell, F. F. *Journal of Chromatography A* **1993**, *656*, 549-576.

(48) James, R. O.; Healy, T. W. *Journal of Colloid and Interface Science* **1972**, *40*, 42-&.

(49) James, R. O.; Healy, T. W. *Journal of Colloid and Interface Science* **1972**, *40*, 53-&.

(50) James, R. O.; Healy, T. W. *Journal of Colloid and Interface Science* **1972**, *40*, 65-&.

(51) Zou, H. F.; Zhang, Y. K.; Lu, P. C. *Mikrochimica Acta* **1991**, *1*, 145-149.

(52) Bartha, A.; Vigh, G. *Journal of Chromatography* **1983**, *260*, 337-345.

(53) Bartha, A.; Vigh, G. *Journal of Chromatography* **1983**, *265*, 171-182.

(54) Snyder, L. R.; Dolan, J. W. In *Advances in Chromatography, Vol 38*, 1998; Vol. 38, pp 115-187.

(55) Bartha, A.; Billiet, H. A. H.; Degalan, L.; Vigh, G. *Journal of Chromatography* **1984**, *291*, 91-102.

(56) Bartha, A.; Vigh, G.; Stahlberg, J. *Journal of Chromatography* **1990**, *506*, 85-96.

(57) Zou, H. F.; Zhang, Y. K.; Hong, M. F.; Lu, P. C. *Chromatographia* **1991**, *32*, 329-333.

(58) Bartha, A.; Stahlberg, J. *Journal of Chromatography A* **1994**, *668*, 255-284.

(59) Kavanau, J. L. *Water and Solute-Water Interactions*; Holden-Day, Inc.: San Fransico, 1964.

(60) Guo, J. H.; Luo, Y.; Augustsson, A.; Kashtanov, S.; Rubensson, J. E.; Shuh, D.; Zhuang, V.; Ross, P.; Agren, H.; Nordgren, J. *Journal of Electron Spectroscopy and Related Phenomena* **2004**, *137-40*, 425-428.

(61) Laaksonen, A.; Kusalik, P. G.; Svishchev, I. M. *Journal of Physical Chemistry A* **1997**, *101*, 5910-5918.

(62) Hawlicka, E.; Swiatlawojcik, D. *Chemical Physics* **1995**, *195*, 221-233.

Chapter 6 Conclusions and Future Work

6.1 Conclusions

The work presented in this thesis is most importantly a study of alternatives. The incorporation of the chemiluminescent nitrogen detector (CLND) into a broad range of HPLC separation techniques clearly requires a revision of the methods used with more conventional detectors. The sensitivity of the CLND proves to be both an advantage and a challenge. It has been demonstrated to be capable of good sensitivity in the analysis of nitrogen compounds in complex matrices this ultimately requires very pure (nitrogen free) eluents. Though this is not likely to be an issue in for industrial reversed phase separations, the manufacturers intended market for the detector. The use of this detector for alternative analysis requires vigilance in the choice of eluents and methodology.

It is clear that the CLND is capable of competing with more complex detection schemes for both cyanide and metal cyanide compounds. Additionally it is capable of direct detection of traditionally difficult analytes, such as zwitterionic surfactants. The specificity of the detector allows for any of the analysis to be done with little fear of interference from the matrix.

Conversely the difficulties in producing a truly nitrogen free eluent for anything other than a reversed phase separation are significant. In addition the detector as a whole has not been very robust when significant amounts of non-volatile material are present in the eluent. This however can be overcome with some of the more modern ion suppression technologies, which previously were not compatible with the CLND.

The challenges that are posed by the limitations of the CLND do prove to be advantageous through the need to return to the fundamentals of the separation. New examinations of the methodologies adopted in the past reveal new options for the present situation. The development of TBP and TBS as valid ion pairing agents is an example of this benefit. The need for an alternative to traditional ammonium ion pairing agents not only revealed new ion pairing agents, it furthered the understanding of ion pair separations. The incorporation of the Hofmeister series into our understanding of ion pair separations should prove invaluable in further advances of both ion pair and ion exchange chromatography.

6.2 Future Work

6.2.1 Further Understanding of IPA Separation

This thesis presented the beginning of a new understanding of ion pair separations which will surely improve chromatographic techniques for ion analysis. The basis of this theory is found in the Hofmeister series and the solvation of ions[1]. The solvation of ions becomes important when one adopts the double-layer view of IPC[2]. The understanding that increases in retention occur due to greater penetration of an ion into the double-layer is based upon the work James and Healy[3-5] on double-layers. The view that dehydrated ions penetrate further into the diffuse layer coincides well with the increases in retention seen for anions in my work.

Yet to further the understanding of IPC a greater knowledge of how the ion-solvent interactions of the analyte can be altered is required. A primary need is a better understanding of the classification of both analyte and IPA in terms of their kosmotropic/chaotropic character. It is clear from the work presented that changes in retention factor are dependant upon the qualities of both the IPA and the anion. This was most clearly seen with the multivalent anions, Section 5.4.1.4, where a number of different results were seen when the IPA concentration was increased. Having a better classification of the characteristics of the analytes and IPAs will allow for the determination of what requirements are needed in order to illicit a selectivity change.

Additionally there are a number of variables left to be examined in IPC, beyond those in the extensive studies by Bartha and Vigh[6-10], which could prove to reveal even more about the retention process. Among these variables are the type of organic modifier. Though there is some knowledge as to the results of various organic modifiers on the structure of water, understandings of the influences upon the water in the nearest regions of the anion are unclear, particular as to how this would impact IPC. It has already been noted by some researchers that better peak shapes are achieved with acetonitrile over methanol[11], yet the reasons for this are unknown.

The impact of altering both the concentration and nature of the buffer salt also needs to be evaluated. As ions can penetrate the L_3 region[1] there can be an impact on the hydrogen bonding structure around the anion. With anions that span the range of kosmotropic and chaotropic influences there is a great potential for modification of the influences of the L_3 bonding structure. As anions have the greatest impact on the Hofmeister scale[1] it is possible that the effects already seen with IPAs could be enhanced with the proper combination of buffer anions. The combination of kosmotropic and chaotropic characteristic from both the IPA and the buffer could allow for greater selectivity control.

Furthermore there are even more IPAs suitable for anion analysis waiting to be studied. These include arsenic and selenium compounds, which have been separated by IPC[12], yet have not be utilized to perform the same separations of anions. As yet there is no clear trend explaining why the specific IPAs impact the separations as is seen. Observation of more IPAs is likely to yield more information regarding what characteristics make an IPA more of less chaotropic. This understanding could allow for the modification of the alkyl chains in order to further enhance kosmotropic or chaotropic characteristics.

Finally temperature is yet another variable to examine. Traditionally temperature has been kept constant to favour reproducible separations[13]. Yet it was shown in the work of Hatsis and Lucy that temperature can effect selectivity changes in ion chromatography[14; 15]. The fact that temperature fluctuations can lead to irreproducible results is further proof that temperature will influence the retention of analyte ions. It would be anticipated that the changes in selectivity for anions in IC would be similar to those seen with ammonium IPAs, as ammonium cation is the primary bonded phase material for anion separations. However, to my knowledge, the influences of temperature on phosphoniums and sulphoniums, offering the potential of further changes in selectivity.

6.2.2 Further Potential of CLND

The CLND is obviously a potent analytical tool which until the development of non-nitrogen containing IPAs was limited to primarily reversed phase separations. Yet with the ability to accommodate TBP and TBS as eluent additives the utility of the CLND has expanded greatly. This thesis has shown that CLND can be used to analyze such inorganic nitrogen species as azide, cyanide and metal cyanide complexes. However the potential for biological and pharmaceutical analysis have not been examined. It has always been known that the CLND is capable of reversed phase analysis of biological and pharmaceutical compounds, however not all compounds of interest can be separated through RPLC. IPC is used frequently for analysis of proteins, peptides and pharmaceutical compounds[16]. Through the use of TBS or TBP these separations can now be performed with CLND. This offers a distinct advantage over other IPC separations as there is no need for a chromophore or other detector specific functionality, as virtually all these compounds contain nitrogen. Additionally with knowledge of the nitrogen content of an analyte the universal response factor of the CLND offers simple quantification of separations. Obviously future work with the CLND should take advantage of the new IPAs in order to improve IPC analysis of biological and pharmaceutical compounds.

It is clear from the work in this thesis that there is much potential room for improvement of our knowledge of HPLC separation techniques. Although solutions have been found to most of the common problems, further advancements of separation requirements will necessitate revaluation of those solutions. Re-examination of solutions to prior problems can yield new incites into the fundamentals of how the separations are achieved and how they can be further manipulated.

6.3 References

(1) Collins, K. D.; Washabaugh, M. W. *Quarterly Reviews of Biophysics* **1985**, *18*, 323-422.

(2) Chen, J. G.; Weber, S. G.; Glavina, L. L.; Cantwell, F. F. *Journal of Chromatography A* **1993**, *656*, 549-576.

(3) James, R. O.; Healy, T. W. *Journal of Colloid and Interface Science* **1972**, *40*, 42.

(4) James, R. O.; Healy, T. W. *Journal of Colloid and Interface Science* **1972**, *40*, 53.

(5) James, R. O.; Healy, T. W. *Journal of Colloid and Interface Science* **1972**, *40*, 65.

(6) Bartha, A.; Billiet, H. A. H.; Degalan, L.; Vigh, G. *Journal of Chromatography* **1984**, *291*, 91-102.

(7) Bartha, A.; Vigh, G. *Journal of Chromatography* **1983**, *265*, 171-182.

(8) Bartha, A.; Vigh, G. *Journal of Chromatography* **1983**, *260*, 337-345.

(9) Bartha, A.; Vigh, G. *Journal of Chromatography* **1987**, *395*, 503-509.

(10) Bartha, A.; Vigh, G.; Billiet, H. A. H.; Degalan, L. *Journal of Chromatography* **1984**, *303*, 29-38.

(11) Giroux, L.; Barkley, D. J. *Canadian Journal of Chemistry* **1994**, *72*, 269-273.

(12) Aced, G.; Mockel, H. J.; Nelsen, S. F. *Journal of Liquid Chromatography* **1989**, *12*, 3201-3218.

(13) Snyder, L. R.; Glajch, J. L.; Kirkland, J. J. *Practical HPLC Method Development*; Wiley: New York, 1988.

(14) Hatsis, P.; Lucy, C. A. *Analyst* **2001**, *126*, 2113-2118.

(15) Hatsis, P.; Lucy, C. A. *Journal of Chromatography A* **2001**, *920*, 3-11.

(16) Hearn, M. T. W. *Ion-Pair Chromatography, Theory and Biological and Pharmaceutical Applications*; Marcel Dekker, Inc.: New York, 1985.